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AMERICAN DAIRY SCIENCE ASSOCIATION

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By action of the Executive Committee the next meeting of the Association will be held at the Iowa State College in June 1930 at a date to be announced.

The Program Committee invites the attention of members to the desirability of making early preparation for the presentation of papers. Suggestions are also invited regarding the character of the program to be prepared. A considerable part of the program will be the presentation of brief reports on research. On account of the large amount of material likely to be offered the committee can not insure the acceptance of all papers offered. Members engaged in commercial work are urged to take a part in the program.

Except by special arrangement each speaker will be limited to 15 minutes. It is expected that not over 10 minutes will be used in the presentation of the material, leaving 5 minutes for discussion. Titles of papers should be in the hands of the committee by March 15th and copies of the material to be presented not later than May 1st. The following suggestions for papers are offered.

1. It is expected that material presented cover research work not already published.

2. The presentation of new experimental methods, new equipment or other unique features is particularly desirable.

3. The presentation should be essentially a statement of conclusions to date, omitting most of the details of the research. In some cases plans for the future would be especially welcomed. The committee commends the practice of using mimeographed sheets to present tables and details.

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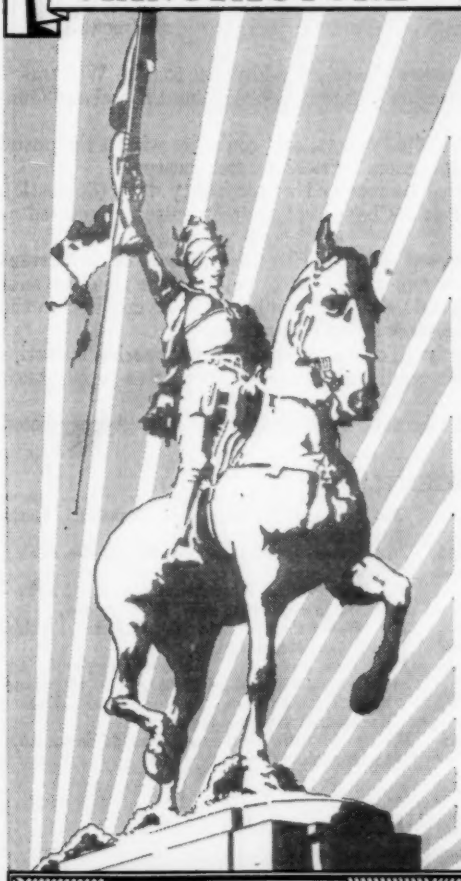

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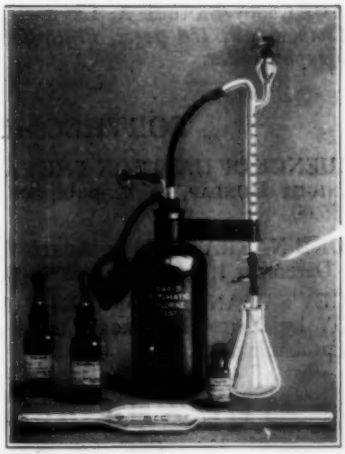

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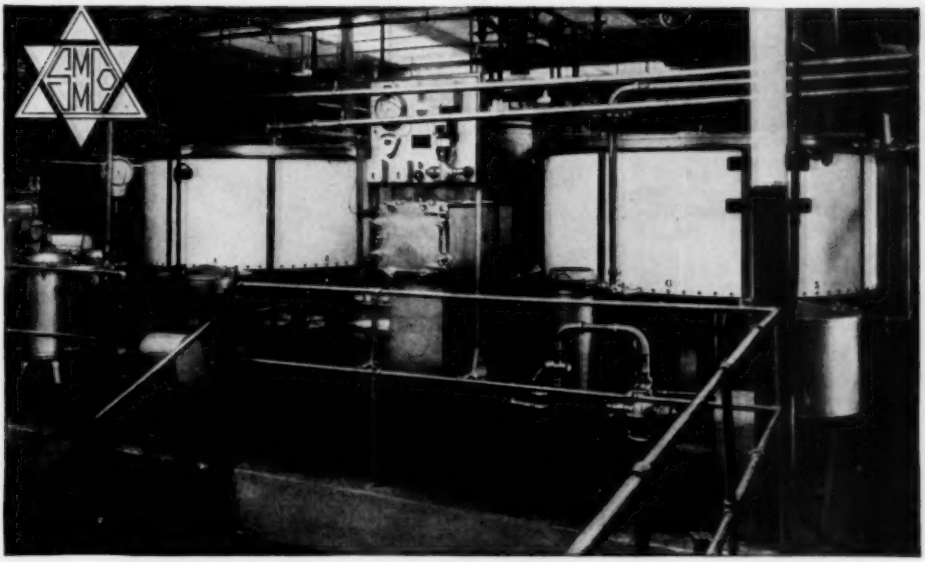

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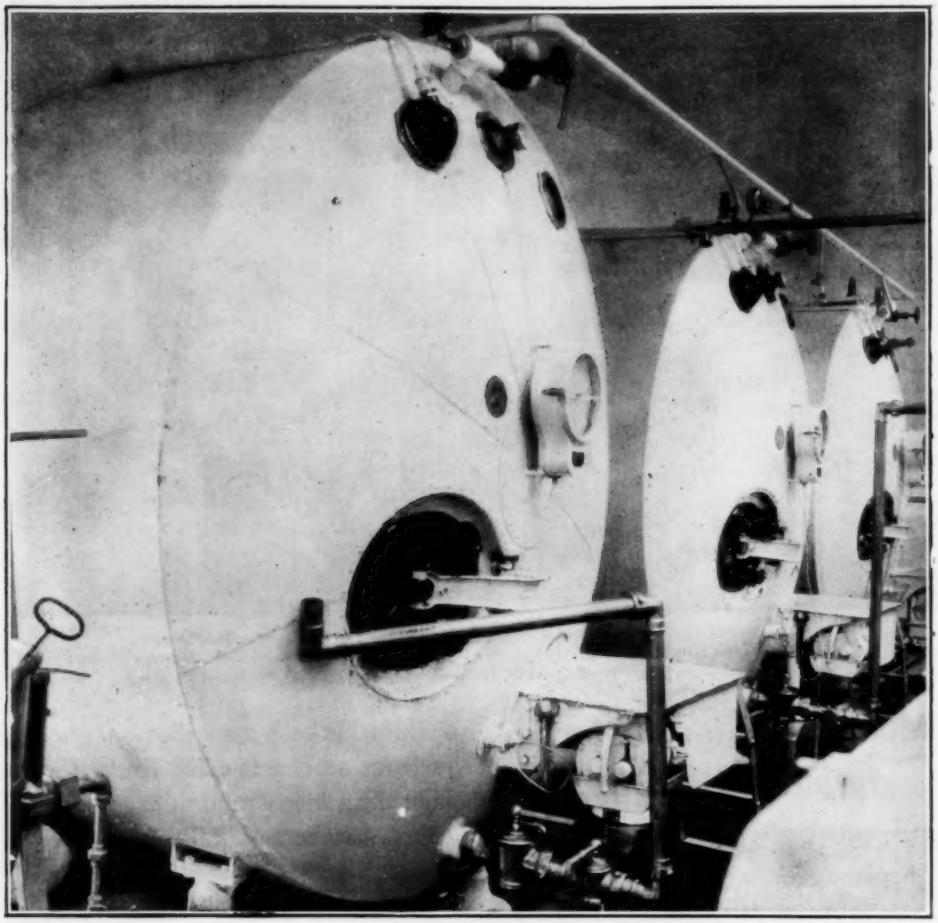

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THE INFLUENCE OF THE USE OF BUTTER ON THE FREEZING PROPERTIES OF ICE CREAM MIX*

RANDALL WHITAKER

Department of Dairy Industry, Cornell University, Ithaca, New York

In some sections of the United States milk fat for ice cream can be purchased more cheaply in the form of butter than in the form of sweet cream. As a result butter is quite commonly used for this purpose and the ice cream produced is said to compare favorably in quality with that containing sweet cream. Clutter (1) and Wright (2) studied some of the factors which influence the freezing properties of ice cream mixes and found among other things that mixes made with butter did not swell as much nor as rapidly as those made with cream. They also observed that butter mixes were more viscous than cream mixes. No adequate explanation seems to have been offered to account for these observations.

This paper records the results of experimental work designed to study the differences in the freezing properties of ice cream mixes made with cream and butter.

EXPERIMENTAL

A quantity of fresh 40 per cent cream was held for several hours at 45° to 50°F. One-half of the cream was then churned and worked into butter. This butter and the remainder of the cream were then used in the preparation of separate mixes, each of which contained 10 per cent fat, 10 per cent M.S.N.F., 14.5 per cent sugar and 0.5 per cent gelatin. Dry skimmilk was used as the source of the solids-not-fat required in addition to those furnished by the cream.

After pasteurization each mix was homogenized at 3000 pounds pressure, cooled and aged for twenty-four hours at 35°F. All mixes were then frozen in a forty-quart brine freezer. The

* Received for publication May 13, 1929. Supported by a grant from the American Dry Milk Institute.

swell and consistency of the ice cream in the freezer were determined at minute intervals. This procedure was repeated, using in all, four lots of cream each of which was from a different source.

A summary of the freezing properties of the mixes made with cream showed that a swell of 90 per cent was obtained 4.5 minutes after the brine was turned off, whereas the butter mixes averaged 8.2 minutes before this selected overrun was reached. An average maximum swell of 101 per cent was obtained with the mixes

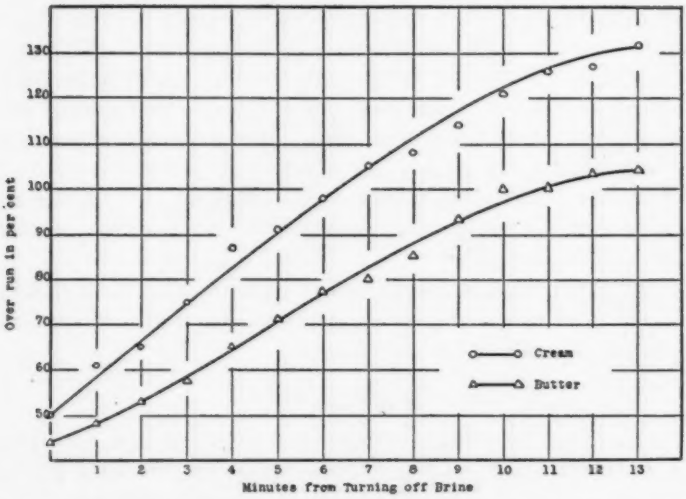

FIG. 1. SWELL DEVELOPMENT OF MIXES MADE WITH CREAM AND WITH BUTTER

containing cream, while the butter mixes had an average maximum swell of 76 per cent. The swell development of both the cream and butter mixes is shown graphically in figure 1. It is evident that the mixes made with butter did not whip as rapidly nor to as high a maximum as the mixes made with cream.

Cream may be considered to be a mixture of butter and buttermilk. In the butter mix the buttermilk portion of the original cream was not incorporated, since it was discarded during the manufacture of the butter. It was necessary then, to increase

slightly the amount of dry skimmilk in the butter mix to maintain the desired serum solid standard of 10 per cent. The cream and the butter mixes differed only in this substitution of dry skimmilk for the buttermilk solids of the cream lost in the churning process. It seems logical to assume therefore, that the unequal freezing properties of these mixes might be attributed either to the quantity of dry skimmilk used or to the amount of buttermilk solids present in each mix. The work of Lucas and Roberts (11) indicates that the amount of dry skimmilk used in a well

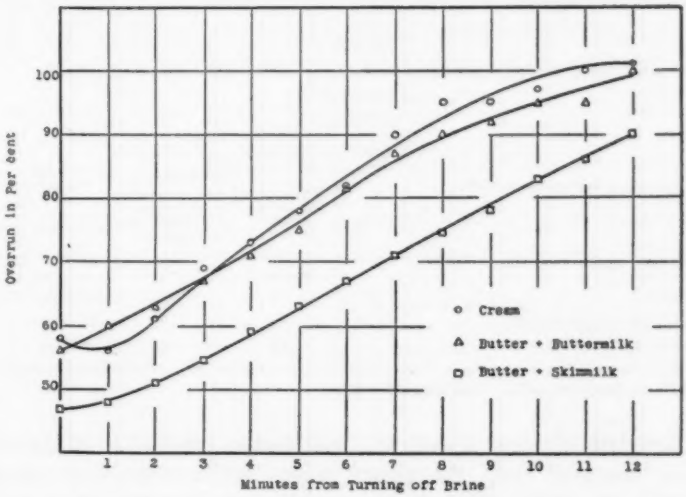

FIG. 2. SWELL DEVELOPMENT OF MIXES MADE WITH BUTTERFAT FROM VARIOUS SOURCES

proportioned mix containing 10 per cent fat has no significant influence on the whipping properties. It would seem, therefore, that the difference noted in the freezing properties was due to the amount of buttermilk solids, rather than due to the quantity of dry skimmilk.

If the more desirable freezing properties of the cream mix could be attributed to the presence of the buttermilk, it would follow that a butter mix would exhibit about the same freezing properties

as a cream mix if the buttermilk produced during churning were incorporated in the mix with the butter. Such a batch was made and compared with one mix containing cream and a second mix made with butter in the manner already described. The milkfat was, of course, obtained from the same source of milk. This procedure was repeated, using in all, three separate lots of cream. The mixes in this experiment contained: fat 12 per cent, M.S.N.F. 11 per cent, sugar 14.5 per cent and gelatin 0.5 per cent. Data concerning the behavior of these three mixes in the freezer are shown in table 1 and in figure 2.

TABLE 1

Freezing properties and basic viscosity of mixes made with and without buttermilk solids

DESCRIPTION OF MIX	FREEZING PROPERTIES		BASIC VISCOSITY OF AGED MIX
	Time to reach 90 per cent swell*	Maximum swell obtainable with desired consistency	
	minutes	*per cent*	*cp.*
Cream	7	92	89
Butter and buttermilk	8	90	83
Butter	12	75	121

* Time from moment of turning off brine.

The data of table 1 indicate that butter mixes had a higher basic viscosity than the cream mixes which contained more buttermilk solids. This is in accordance with the statements of Clutter (1) and Wright (2). It is also apparent that the freezing properties of the mix in which the buttermilk solids were absent were inferior to those of the mixes containing these solids. It would seem from these results that the coalescing of the fat globules during the churning process and the subsequent reëmulsification of the fat in making the ice cream had but little effect on the swell development. Apparently buttermilk and cream contain some factor which is wholly or partially lacking in butter which is able to affect to a marked extent the viscosity and the freezing properties of the mixes.

DISCUSSION

Clutter (1) and others observed that high basic viscosity is often associated with low overrun and that an important factor in determining basic viscosity is the extent of the aggregation of the fat globules. These results have been confirmed by the experiments reported here and by work as yet unpublished by the author. Examination of the mixes made in the course of these experiments indicated a marked difference in the clumping of the fat globules. The fat in those mixes containing more of the buttermilk portion of the cream did not gather in large aggregations during the homogenizing process, but this clumping did occur in the other mixes. This suggests the presence of a

TABLE 2

Lecithin in milk products

MATERIAL	AVERAGE PERCENTAGE OF LECITHIN
Milk	0.0447
Cream	0.1981
Skimmilk	0.0165
Buttermilk	0.1302
Butter (raw sweet cream)	0.0723
Butter (pasteurized ripened)	0.0433

material capable of stabilizing the emulsion of the fat in those mixes containing the buttermilk portion of the cream.

Examination of the available information brings out the fact that this material might be lecithin. Table 2 from the work of Chapman (3) and Supplee (10) shows that buttermilk and cream contain 13 to 20 times as much lecithin as skimmilk. Palmer and Samuelson (4) found that the butter milk from washed cream contained a mixture of phosphatides. Thurston and Petersen (5) present further evidence in support of this theory. They determined the lecithin content of buttermilk made by churning creams of various fat contents and showed that the richer the cream in fat the greater the quantity of lecithin found in the buttermilk. Dornic and Daire (6) suggest that lecithin is asso-

ciated with the butterfat, probably in the form of a film around the fat globules, and that the coalescing of the fat globules during the churning process brings about the mechanical separation of the lecithin from the fat, causing it to remain in the buttermilk.

The probable difference in the lecithin content of the mixes with and without the buttermilk portion of the cream suggests that lecithin may be the factor causing the marked difference in the freezing properties.

The theory that the presence of lecithin in ice cream mix improves the whipping quality, is further supported by the statements of Dahle (7), Turnbow and Raffetto (8), Wright (2) and others, that the addition of dry egg yolk to the mix has a beneficial effect on the swell. Dahle says that egg yolk "is a good emulsifying agent and a very small amount will stabilize a large amount of fat." According to Haas and Hill (9) egg yolk contains 9.4 per cent of lecithin.

SUMMARY

1. Experimental data is presented to show that the use of butter in place of cream as the source of fat in ice cream mixes produces a mix of inferior freezing qualities.

2. Butter mixes differ from cream mixes in that they do not contain the cream serum or "buttermilk solids" of the cream.

3. Data recorded by previous workers indicate that the lecithin content of cream and buttermilk is considerably higher than that of skimmilk and butter.

4. It is suggested that the lecithin incorporated in ice cream with cream, buttermilk or egg yolk may be responsible for the differences in the freezing properties observed when this material is partially lacking in the mix.

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THE PHYSIOLOGICAL EFFECT OF PITUITARY EXTRACT (POSTERIOR LOBE) ON THE LACTATING MAMMARY GLAND*

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Extracts of the pituitary gland (hypophysis cerebri) have long been known to have an effect on the blood pressure (Oliver and Schafer (19)) inducing a marked rise following injection. Subsequently there was observed contractile action on smooth muscle and a diuretic effect. Howell (13) first pointed out that the active principal was a constituent of the posterior lobe. Herring (12) however, produced evidence indicating that while the active principal is present in the posterior lobe, it is a product of the cells of the pars intermedia.

The profound effect on the lactating mammary gland of infundibulin (the active principle of the posterior part of the hypophysis) was first demonstrated by Ott and Scott (20) in 1910. Upon the injection of an extract into a lactating goat, the flow of milk started in about one minute, reached its maximum in four minutes, and then fell rapidly. These results were quickly confirmed with lactating cats and dogs by Schafer and Mackenzie (22), in dairy cattle by Gavin (7), and in man by Schafer (23).

In cattle Gavin (7) observed a rapid flow of milk to the lower parts of the udder, the teats usually being distended within ten to fifteen minutes of injection. No change was noted in the daily yield or composition of the milk.

Schafer and Mackenzie (22) noted that the effectiveness of repeated doses was greatly reduced or entirely absent. Hammond (10) verified this observation and tried without effect to overcome the supposed immunity by enlarged doses. Differing from Gavin's results, Hammond observed an increase in the percentage

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of fat in the milk following the injection. Simpson and Hill (25) working independently, confirmed the observations of Hammond.

All investigators are agreed that the injection of extracts from the posterior lobe of the pituitary brings about a rapid flow of milk when the teat has been excised or makes immediately available a certain quantity of milk which could not ordinarily be removed by milking or nursing.

There is, however, a considerable difference of opinion in regard to the mode of action. It was thought by the early workers that pituitrin was a true galactogogue stimulating the synthesis of milk. Others claimed that the extract acted on the mammary gland in some manner resulting in the expulsion of the formed milk from the individual secreting cells or from the alveoli or both.

Experimental evidence in favor of the secretory theory of action was advanced by Simpson and Hill (25) who injected barium chloride, a strong smooth muscle stimulant, into a goat and found no increased milk flow. They conclude, therefore, that the action is on the secretory mechanism of the gland. Maxwell and Rothera (17) found that the injection of the extract into goats which had only recently been milked dry caused considerable additional milk to become available. They state, "It seemed to us unlikely that such a large increase could be due to simple pressure on the gland caused by contraction of its muscular element." Further, it was shown that following an injection there was an increased milk pressure lasting seventeen and one-half minutes in a goat and at least forty minutes in a cow. They conclude that the effect of pituitrin is the same as suckling causing a true secretion of milk. More recent work which will be described later indicates that little if any actual secretion of milk occurs as a result of suckling or milking.

Hammond (10) concluded as a result of his work that "an injection of pituitary extract is to cause the immediate secretion of the milk (stored in the gland) which would otherwise have been drawn off at the next milking." Schafer (23) gives the following observation of his patient "that though there was more milk than

usual for the baby for the meal following the injection, I had to wait a long time before I could feed him again."

The most pertinent objection to the secretory theory is advanced by Schafer (24). He pointed out that a second injection administered immediately after a gland has been completely emptied is without effect. It is then quite impossible to get a drop of milk from it, although if the action is on the secreting mechanism a free flow should be obtained. To produce the reaction one must either use a gland which is full or one not completely depleted; and if it has been depleted, one must wait until some secretion has accumulated within the alveoli.

MODE OF ACTION

Several theories have been advanced in explanation of the mode of action of pituitrin.

It has been suggested that the mode of action is through alterations produced on the circulation of blood. It was shown by Howell (13) that injections of the extract slowed the heart rate but increased the blood pressure. Both effects are less marked than in the case of epinephrin (extract of the adrenal medulla) but they persist for a longer time. Geiling (8) states further that the respiratory rate is quickened, but is interspersed with periods of cessation of breathing in unanesthetized dogs. Repeated injections have not only little or no blood pressure-raising action, but may produce a diametrically opposite effect—a depressor response.

A further action of the extract is its stimulating effect on most of the smooth muscles of the body. The intestine and bladder are made to contract. There is also considerable oxytocic activity through the stimulating effect upon the uterine musculature. The difference in the mode of action of pituitary extract and epinephrin is described by Howell (14). "While epinephrin acts mainly at least on plain muscle innervated by the sympathetic autonomics and gives contractions or relaxation according as the nerve-fibers are motor or inhibitory, the hormone from the posterior lobe of the pituitary gland appears to act directly on the muscle and to cause contraction or increased tone in all cases."

Because of the general similarity of some of the physiological reactions of pituitary extract and epinephrin, the comparative action on the lactating mammary gland is of extreme interest. Hammond and Hawk (11) found that following injections of adrenalin (epinephrin) in the goat there was no immediate action on milk secretion, whereas there was a secondary effect indicated by a decrease in the amount of milk produced for a period of a day following its injection.

Similarly Simpson and Hill (25) injected barium chloride, a strong muscle stimulant, into a goat and found no increased milk flow.

The absence of stimulation of milk flow with these extracts which are known to react upon smooth muscle immediately raises the question as to the presence of smooth muscle or related structures surrounding the alveoli or ducts. Unfortunately this problem is still in a contraversial state with little general agreement. Benda (1) found in the club shaped ends of the gland tubules between the low cuboidal epithelium and the membrana propria, cells which he considered identical to the muscle cells of the sweat glands. He was of the opinion that these structures possess contractility and play a part in the physiology of secretion. Zacher (30), Unger (29), Spampani (27), Lacroix (15), Christ (3), Lenfers (16), Bertkau (2) and others maintain that they have observed either smooth muscle cells, or basket cells (myoepithelium) which have contractile properties associated with the alveoli and ducts of the mammary gland.

On the other hand, Hammond (10) and more recently Dieckmann (4) advance the idea that the cells thought to be myoepithelium are, instead, cells which surround the capillaries of non-epithelial origin.

Instead of a muscular action Hammond (10) believes that the pituitary extract acts directly upon the glandular epithelium causing the contraction of the cells and expulsion of the secretion. Sections of the gland following pituitary extract injection showed alveoli distended with milk and the epithelium cells flattened. The epithelial cells of the control were columnar and protruded into the lumens, which were small, and contained only limited

quantities of milk. In a few places, however, the alveoli were filled, and here the epithelial cells were flattened.

In spite of the work of Simpson and Hill (25) showing that barium chloride had no effect on the mammary gland Schafer (24) still maintains that the "complete emptying of a gland which obtains after an adequate injection can best be explained by supposing the existence of contractile tissue in the walls of the alveoli."

In support of this theory Schafer presents a microphotograph showing the alveoli of two adjacent mammary glands of a lactating cat. In one of these the nipple had been excised so that the milk could flow away freely, in the other gland the nipple was left intact, the resistance furnished by its tissues being always sufficient to prevent an outflow of secretion under these circumstances. After injection, the alveoli in the gland with the excised teat showed small irregular lumens, whereas the others were expanded and full of milk. He concludes, "It seems to me impossible to suppose that the vis a tergo produced by the action of the secreting cells in forwarding the fluid into the alveoli could produce such a picture as that of the emptied gland here exhibited."

A further hypothesis as to the mode of action of pituitrin is one suggested to Schafer (24) by Professor T. H. Milroy. It is postulated that the alveoli are highly extensible and elastic; that the secretion accumulates within them owing to a tonic contraction of plain muscle in the ducts, preventing its exit; and that pituitrin produces inhibition of this contraction so that elastic reaction of the alveoli can produce the outflow which is then obtained. The result of the experiment with barium which Simpson and Hill describe would fit in with this inhibitory hypothesis, while it appears to contraindicate the contraction hypothesis.

Schafer maintains that the emptying of the alveoli due to retraction of stretched elastic fibres in their walls, consequent on inhibition of plain muscle in the ducts is ingenious, but receives no support from analogy; nor has it been shown that the mammary gland is better provided with elastic fibres than other secreting glands. In fact, in the cat and rat gland there are

practically no elastic fibres in the walls of the alveoli nor between the alveoli, and these fibres are not especially numerous in the interstitial tissue of the gland.

Evidence from another source in favor of the above hypothesis is presented by Emmerson (5) who injected a 2 per cent solution of apothesin (a local anasthetic) around the inguinal nerves of a lactating dairy cow. In one case it was noted that although the cow was milked but a few hours previously, milk began to drop from the ends of the teats for a period of 45 minutes. In a second case, a cow producing 21.5 pounds of milk daily was remilked twenty minutes after an injection and produced 3.7 pounds of milk.

OBJECT OF THE INVESTIGATION

The review of experimental work indicates that pituitrin is one of the few animal extracts which has a definite physiological effect on the mammary gland. It, therefore, becomes of great importance in connection with studies on milk secretion and the physiological activity of the mammary gland during the milking process.

The discharge of milk at the time of milking noted by Nüesch (18) Maxwell and Rothera (17), and Tgetgel (28), as indicated by an increase in pressure within the cistern system, appears to be the result of a nervous reflex. The stimulus of milking usually sets in motion the phenomena called "letting down" of the milk extensively studied by Tgetgel (28). The nervous stimulus may be brought to the gland through the activity of motor nerves acting on the smooth muscles. It is possible, however, that one of the normal functions of the pituitary gland, which is so closely connected with the nervous system, is to regulate the discharging phase of milk secretion. If this were the case, the nerve paths would lead to the pituitary gland causing the discharge of the hormone which would in turn bring about the well known change in the gland.

PRESENTATION OF EXPERIMENTAL DATA

Our first objective in beginning experimental work with pituitrin[1] was to standardize the extract in terms of its physio-

[1] Lilly's Pituitary Extract, Surgical, Double U. S. P. strength was used.

logical activity in the lactating dairy cow. The statement of Hammond (10) that the extract causes the immediate secretion of the milk stored in the gland was thought to offer such a standard. The plan adopted was to milk the cow regularly at 5 a.m. and 5 p.m. each day. On Monday and Wednesday of each week the cow was remilked at 5:30 p.m. On Friday immediately after the regular milking 2 cc. of the extract were injected subcutaneously. The cow was then remilked at 5:30 p.m. Each week the volume of extract was increased by 2 cc.

TABLE 1

The effect of varying amounts of pituitrin

PITUITRIN	30 MINUTES REMILK (2 DAY AVERAGE)		30 MINUTES REMILK AFTER INJECTION		12 HOURS MILKING AFTER INJECTION		24 HOURS MILKING AFTER INJECTION	
	Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat
cc.	*cc.*	*per cent*	*cc.*	*per cent*	*pounds*	*per cent*	*pounds*	*per cent*
2	101.0	5.0	233.0	5.45	12.0	3.0	13.4	3.1
4	93.5	4.7	504.0	5.90	10.0	2.4	14.2	3.1
6	85.0	5.4	870.0	6.10	9.5	1.6	16.0	3.6
8	123.3	6.0	812.0	7.10	7.5	1.9	15.7	3.4
10		7.2	803.0	7.10	5.2	1.4	15.3	2.8
12		6.0	916.0*	7.90	4.7†	6.0	15.6	3.1

* Milked at ten-minute intervals for thirty minutes.
† Milked hourly for six hours following injection.
440 cc. of milk is equal to 1 pound.

The effect of increasing amounts of pituitrin on the amount of milk which could be obtained thirty minutes after the previous milking is shown in table 1 and figure 1. It will be seen that the yield of milk increases until 6 cc. were injected. Slightly more milk was obtained with 12 cc., but in this case the cow was milked at ten-minute intervals for thirty minutes. The experimental animal weighed about 1200 pounds. From these observations it is concluded that approximately 0.5 cc. of pituitrin per 100 pounds live weight produces the maximum physiological effect so far as the expulsion of milk is concerned.

The percentage of fat shows a gradual rise as the amount of extract increases.

TEMPORARY INHIBITION OF MILK SECRETION

Not only does pituitrin exert an immediate influence on the gland following an injection, but there appears to be a secondary effect on the subsequent secretion of milk much greater than that which can be accounted for by the milk immediately removed. It will be noted from figure 2 that the amount of milk and the

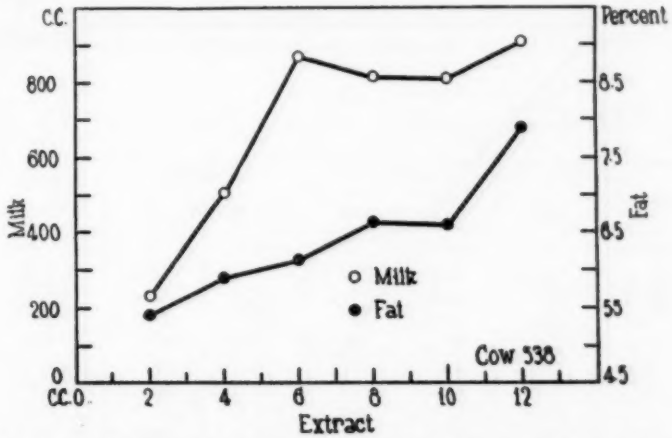

FIG. 1. THE EFFECT OF INCREASING AMOUNTS OF PITUITRIN ON THE AMOUNT OF MILK WHICH COULD BE OBTAINED THIRTY MINUTES AFTER THE PREVIOUS MILKING

The maximum yield of milk was obtained with 6 cc. The percentage of fat continued to increase with the larger injections. The animal weighed about 1200 pounds and was producing about 30 pounds of milk per day. From these observations, it is concluded that 0.5 cc. of pituitrin per 100 pounds live weight produces the maximum physiological effect in forcing out the residual milk from the udder.

percentage of fat obtained twelve hours after the injection decreased as the volume of pituitrin was increased. This phenomena can be explained on the basis that the extract inhibits the normal secretion of milk for a time following its injection, and that the duration of the period is in proportion to the size of the dose. At the rate of decline observed, it would appear that about 18 cc. of extract should entirely inhibit milk secretion for twelve hours.

That the inhibition is temporary, however, is indicated by the yield and composition of the milk obtained twenty-four hours after the injection. At that time the normal yield was obtained in every case. In this connection it may be of some interest to note that the monthly rate of milk secretion was distinctly on the decline as the animal was in the eighth month of lactation when the injections were begun. The monthly milk yield (thirty-one-

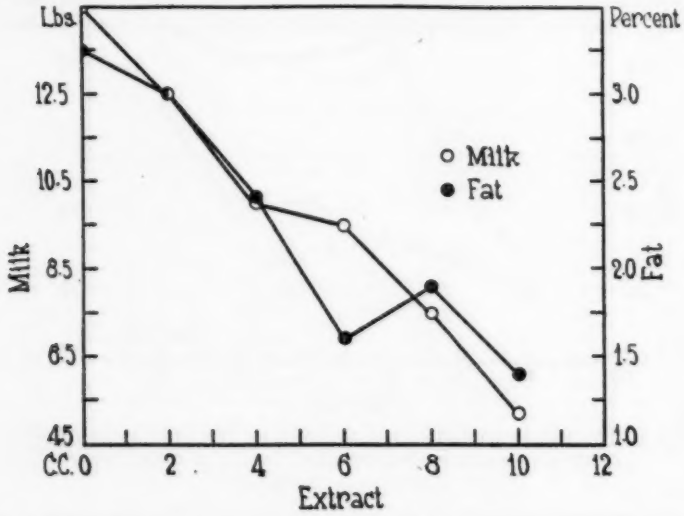

FIG. 2. THE EFFECT OF INCREASING AMOUNTS OF PITUITRIN ON THE AMOUNT OF MILK OBTAINED TWELVE HOURS AFTER THE TIME OF INJECTION

It will be noted that both the yield of milk and the percentage of fat decline, as the dose increases. These results may be explained by assuming that the extract inhibits the normal secretion of milk for a time following its injection.

day month) beginning in May, 1927, were as follows: May, 1244 pounds; June, 1360 pounds; July, 1408 pounds; August, 1318 pounds; September, 1111 pounds; October, 932 pounds; November, 835 pounds. The first injection was made on December 23. The total production for December was 833 pounds, for January 881 pounds (the maximum injections were made at this time) and for fifteen days in February at the rate of 862 pounds of milk.

During the entire period of injection covering approximately two months, the monthly production was maintained approximately at the rate of secretion at the time the experiment was started.

To further study the inhibitory effect of pituitrin, the cow was injected with 12 cc. of pituitrin and milked at hourly intervals for a period of 6 hours. The results are shown in table 2 and figure 3. For comparison, the normal hourly secretion of milk during the two preceding check periods are included.

TABLE 2

The effect of pituitrin injections on subsequent hourly milkings

TIME	NORMAL HOURLY SECRETION—CHECK I		NORMAL HOURLY SECRETION—CHECK II		12 CC. PITUITRIN AFTER 5:30 MILKING	
	Milk	Fat	Milk	Fat	Milk	Fat
p.m.	*cc.*	*per cent*	*cc.*	*per cent*	*cc.*	*per cent*
5:30	6,600	3.50	6,644	3.50	6,292	2.90
6:00	113	5.90	96	6.10	916	7.80
7:00	55	3.00	56	3.70	218	3.40
8:00	575	2.80	398	2.85	225	3.10
9:00	250	2.70	937	3.20	210	2.90
10:00	1,200	2.95	348	3.05	193	2.80
11:00	62	2.65	342	2.75	146	2.25
12:00	850	2.25	743	2.55	151	1.85
Total (excluding first remilk period.	2,992		2,824		1,143	
Grand total..	3,105		2,920		2,059	

Under normal conditions the fluctuation in the yield of milk obtained at hourly intervals is rather large due, it is believed, to the fact that after short intervals the stimulus of milking does not produce the usual pressure changes in the udder which permits the removal of the milk present.

It will be noted that after pituitrin is injected, the normal mechanism regulating the discharge of milk is no longer effective and the milk appears to become available to the milker as it is secreted. From these data it appears that the effect of pituitrin on the mammary gland is to greatly inhibit the rate of secretion.

The fact that the inhibition is rather uniform throughout the entire period is contrary to our expectation. It was believed that little or no milk would be secreted for the first several hours

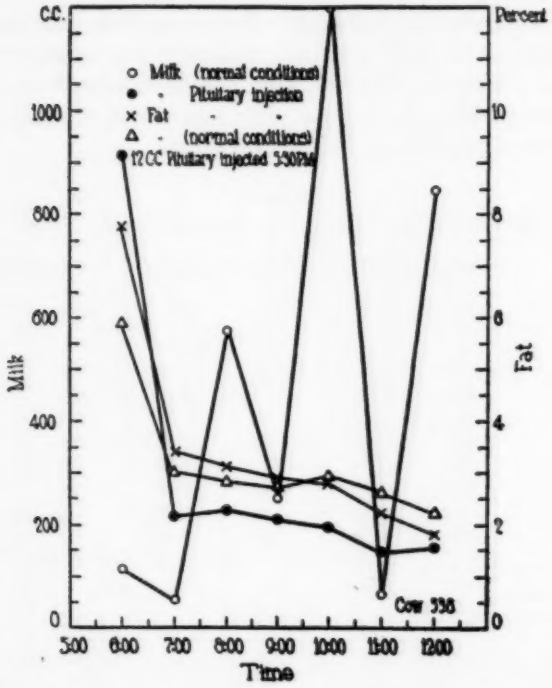

FIG. 3. THE EFFECT OF 12 CC. OF PITUITRIN ON THE YIELD AND COMPOSITION OF MILK DURING THE FOLLOWING 6 HOURS IS SHOWN IN COMPARISON WITH THE NORMAL PRODUCTION

It will be noted that under normal conditions, there are large fluctuations in the hourly production of milk whereas following the injection of the extract the rate of secretion is greatly inhibited but the violent fluctuations are absent.

with a gradual increase as the effect of the extract wore off. On the contrary, there was a gradual decline in the milk yield from first to last. It is possible that the removal of milk may give a false picture of the nature of the inhibition.

The percentage of fat declined rather uniformly in both the normal and experimental periods, but was the more marked after the injection.

In a further effort to determine the length of time the extract inhibits milk secretion, the cow was milked for eighteen hours following an injection. The effect of an injection of 10 cc. of pituitrin on the hourly milk production is presented in

TABLE 3

The length of time the udder was affected by pituitrin

TIME	MILK	FAT	SPECIFIC GRAVITY
a.m.	cc.	per cent	
5:30	6,820.0	4.05	1.0320
6:30	530.0	6.25	1.0250
7:30	313.0	3.15	1.0282
8:30	238.0	2.05	1.0315
9:30	130.0	2.15	1.0319
10:30	88.0	1.80	1.0341
11:30	54.0	2.25	1.0346
p.m.			
1:30	105.0	1.50	1.0370
2:30	252.0	1.75	1.0374
3:30	495.0	1.15	1.0384
4:30	462.0	0.95	1.0385
5:30	1,880.0	2.55	1.0366
6:30	70.0	2.05	1.0345
7:30	134.0	2.25	1.0339
8:30	94.0	2.05	1.0349
9:30	1,040.0	1.95	1.0376
10:30	176.0	1.85	1.0352
11:30	230.0	2.25	1.0358

table 3. The effect was apparently lost in about nine hours, as the variability in the hourly yields of milk noted in previous control milkings was again noticeable at this time. It will be noted that at 5:30 a.m. at the usual milking time, a large yield was obtained.

REACTION TIME

In order to ascertain the time required for the subcutaneous injection of pituitrin to become effective, the cow was remilked at

10 minute intervals for thirty minutes following injections of 6 and 12 cc. of the pituitrin. The results are shown in table 4.

When 6 cc. of pituitrin were injected, the largest amount of milk was available at the end of twenty minutes. With the injection of 12 cc., the largest amount was available at the end of ten minutes. The highest percentages of fat were obtained in the first and second intervals.

TABLE 4

Time required for pituitrin to take effect

AMOUNT OF PITUITRIN	5:30 TO 5:40		5:40 TO 5:50		5:50 TO 6:00		TOTAL
	Milk	Fat	Milk	Fat	Milk	Fat	
cc.	*cc.*	*per cent*	*cc.*	*per cent*	*cc.*	*per cent*	*cc.*
6	133	5.7	375	5.5	150	4.9	658
12	660	8.1	155	8.2	101	6.4	916

COMPOSITION OF MILK

The change in the percentage of fat in the milk following pituitrin injection has already been presented. Complete analysis of a number of samples of milk have also been made. The results are shown in table 5. The "normal milk" refers to the milk obtained at the usual milking period, the milk at "remilking" is that obtained thirty minutes later as a normal check. The influence of pituitrin on the composition will be noted in the comparison with the "remilk" samples.

The results obtained confirm the observations of Hammond (10), and Simpson and Hill (25), namely, that pituitrin affects chiefly the fat content of the milk.

DISCUSSION OF RESULTS

These experiments confirm the work of previous investigators in that pituitrin has been clearly demonstrated to have important physiological properties in connection with the discharge of milk from the mammary gland. Whether the posterior lobe of the pituitary gland plays a part in the normal discharge of milk at milking time is still an open question. Our work was concerned with the effect of increasing amounts of pituitrin causing a

discharge of the "residual" milk in the udder after the normal milking period. That pituitrin does not cause a secretion of milk but rather a discharge of milk already formed in the udder, is indicated by the work of Gaines and Sanmann (6) and Gowen and Tobey (9) who found more lactose in the udder of the cow at milking time than was obtained at a given milking. The average excess of lactose found by Gowen and Toben represented an

TABLE 5

Composition of milk as affected by pituitary extract (cow 538)

DESCRIPTION OF SAMPLE	TOTAL SOLIDS	ASH	LACTOSE	TOTAL PROTEIN	CASEIN	FAT
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cen*
Normal milk January 4, 5:30 p.m.	11.37	0.40	3.71	3.41	2.46	2.7
Milk at remilking 6:00 p.m.	12.09	0.35	3.35	3.13	2.23	4.9
Normal milk, January 6, 5:30 p.m.	11.72	0.50	4.18	3.22	2.62	2.8
Milk, 6 cc. pituitrin 6:00 p.m.	13.65	0.48	4.12	2.87	2.04	6.1
Normal milk, January 9, 11, and 13, p.m.	13.05	0.69	4.71	3.32	2.52	3.3
Milk at remilking, January 9 and 11	15.16	0.67	4.09	3.32	2.39	6.3
Milk, 8 cc. pituitrin, January 13	15.41	0.67	4.07	2.55	1.79	7.1
Normal milk, January 14	11.40	0.72	4.07	3.67	2.84	1.9
Normal milk, January 16 and 18	12.36	0.73		3.28	1.53	3.5
Milk at remilking, January 16	15.40	0.61	4.33	3.00	2.36	7.6
Milk at remilking, January 18	14.55	0.62	3.21	3.06	1.40	6.8
Normal milk, January 20	14.55	0.62	3.21	3.06	1.40	6.8
Milk at remilking, 10 cc. pituitrin January 20	15.69	0.67	4.12	3.03	1.85	7.6
Milk, January 21, a.m.	10.61	0.67	4.23	3.57	2.86	1.4

The chemical analyses were made by the Department of Agricultural Chemistry.

average equivalent of 2.1 pounds of milk remaining in the udder after the cow's udder was believed to be milked dry.

The 4 trials using from 6 to 12 cc. showed that an average of 1.93 pounds of milk were obtained as a result of pituitrin injection. These facts are believed to offer further evidence that pituitrin is not a true galactogogue stimulating the synthesis of milk but rather acts upon the discharging mechanism making it possible to obtain the "residual" milk in the cow's udder.

If pituitrin acted only to cause a more complete discharge of

milk, it would be expected that at the subsequent milking, the yield of milk would be reduced an equivalent amount. These data show, however, that as the amount of extract increased there was a greater and greater reduction in the yield of milk twelve hours later, far exceeding the amount obtained at the remilking periods.

These data indicate a second important physiological property of pituitrin never before demonstrated, namely a temporary inhibition of milk secretion varying in intensity with the amount of extract injected. Subsequent hourly milking indicated that the inhibition of milk secretion is not complete during the first few hours even when sufficient pituitrin is injected to reduce the total twelve-hour milk yield to one-third of that of the normal production. The temporary decrease in milk secretion may be due to a reduction in the flow of blood through the udder due to a contraction of the vascular system. Another possibility is that the secretion of milk may be inhibited by the pressure in the udder due to the contraction of the smooth muscle elements. If the later explanation were correct, the withdrawal of milk at hourly intervals would not give a true picture of the inhibition. A better measure of the effect would be the measurement of the pressure at hourly intervals without removal of any milk. Further observations are required to settle this point.

Reviewing all the evidence, the writers are inclined to the theory that pituitrin is not a galactoguge but rather acts on the mechanism normally effective during the milking process. The excessive amounts injected make possible the discharge of a certain additional amount of "residual" milk still present in the udder at the close of the normal milking act. While the work presented has not contributed to the question of the mode of action of pituitrin or of the normal discharge of milk, we are inclined to believe that contractile elements in the walls of the alveoli and ducts furnish the vis a tergo observed.

SUMMARY

1. The maximum physiological effect indicated by a discharge of "residual" milk was obtained by approximately one-half cubic centimeter of pituitrin per 100 pounds live weight of the cow.

2. A temporary inhibition of milk secretion which varied in intensity with the amount of pituitrin injected was indicated by the yield of milk obtained at the next regular milking.

3. The inhibitory effect of 10 cc. of pituitrin lasted approximately ten hours.

4. The time required for the pituitrin to become effective varied with the size of the injection. The greatest yield of milk was obtained at the end of ten minutes when 12 cc. were injected whereas twenty minutes were required when only 6 cc. were used.

5. The data confirm the work of previous investigators in indicating that probably fat alone of the constituents of milk is influenced by this extract.

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COLOR OF EVAPORATED MILKS*

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The color of evaporated milk is of considerable commercial importance since it is one of the fundamental characteristics by which the consumer judges the product. Some of the various factors concerned in the production of color are recognized in a general qualitative way by the producer. No data, however, are available dealing with the factors concerned in the production of color of evaporated milks from a quantitative standpoint.

It has been the purpose throughout this work to study the effect of various steps in the manufacturing process upon the color of the resulting product, using therein for comparisons, standards of exact known color values.

THE MUNSELL COLOR SYSTEM

The Munsell (2) color system, based upon the psychological fact that color has three attributes; namely, hue, brilliance, and chroma, has been described by Cleland (1), and by Nickerson (3). Troland (4) defines these terms as follows: "Hue is that attribute of certain colors in respect of which they differ characteristically from a gray of the same brilliance and which permits them to be classed as reddish, yellowish. greenish or bluish." "Brilliance (or value) is that attribute of any color in respect of which it may be classed as equivalent to some member of a series of grays ranging between black and white." "Chroma (or saturation) is that attribute of all colors possessing a hue which determines their degree of difference from a gray of the same brilliance." Figure 1 illustrates the relationship of the three color attributes.

Hue. Hue is indicated by the horizontal radii of the color solid and is divided into five principal hues, red (R), green (G), yellow (Y), etc., and five intermediate hues, yellow-red (YR), green-

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yellow (GY), etc. These ten are known as standard hues and each is further subdivided into ten hues.

Brilliance. The vertical axis of the color solid, as shown in figure 1, consists of gradations from white to black through a series of grays. For each position of the hue circle in relation

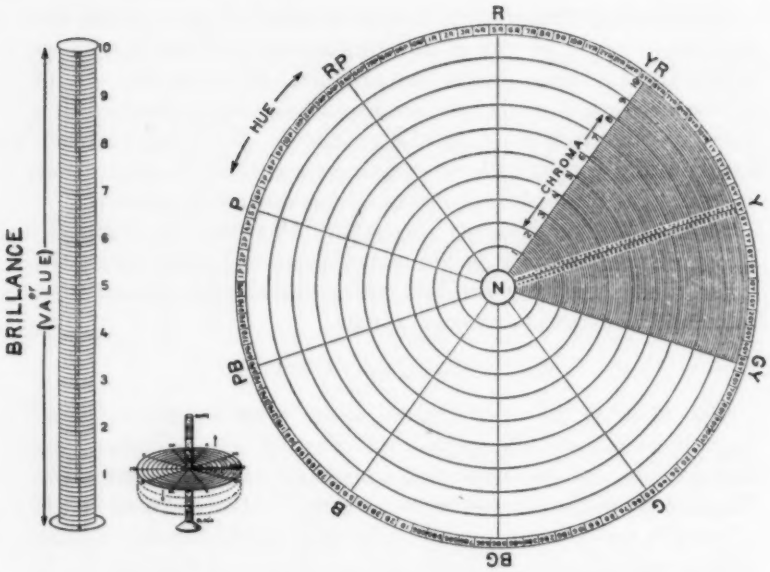

FIG. 1. RELATIONSHIP OF BRILLIANCE, CHROMA, AND HUE ACCORDING TO MUNSELL

to the brilliance scale (small diagram in figure 1) the brilliance of the colors varies.

Chroma. Chroma, or saturation, is indicated on the radii of the color solid, ranging from gray of a certain brilliance at the center to maximum color strength at the circumference. In recording data, brilliance and chroma are usually written as a fraction: Brilliance / Chroma, 4/5, 6/8, etc.

APPARATUS

The Munsell apparatus is provided with discs made to represent the principal and intermediate hues as yellow, yellow-red, etc., at each step in chroma and brilliance. Each disc is marked as to notation of color attributes. The index also provides discs ranging from black to white.

Two or more hue discs and neutral discs are spun on a motor shaft at a speed high enough to combine the colors into a single color. A surface, the color of which is to be measured, is placed beside the discs. A slit in each disc from circumference to center (Maxwell discs) allows for overlapping of the discs and the proportions of the visible sections can be adjusted until there is a perfect match between the object under test and the resolved color. Constant lighting conditions are essential for accurate work. If standard artificial daylight is not available, then North light is next to be preferred. The distance from the eye to the discs should be about 4 feet and the areas exposed to the eye should be equal.

DATA

After the colors are matched the composite disc is superimposed on a disc with a perimeter graduated into 100 divisions and readings of the exposed areas of each disc used are taken. The sum of the hue disc and the neutral discs should equal 100.

The following formulae have been developed for general use in calculating hue, brilliance, and chroma (3):

$$\text{Hue: } z - \frac{\Sigma (A_z P_z)}{\Sigma (A_z P_z) + \Sigma (A_x P_x)} (z - x)$$

$$\text{Brilliance: } \sqrt{\frac{A_1 B_1^2 + A_2 B_2^2 + \ldots\ldots}{100}}$$

$$\text{Chroma: } \frac{A_1 C_1 + A_2 C_2 + \ldots\ldots}{100}$$

where A = area, H = hue, B = brilliance, C = chroma, x = 1st hue, clockwise, z = 2nd hue, clockwise, P = Power ($B \times C$). Notation: $H^B/_C$.

EXPERIMENTAL

Color standards

A normal evaporated milk was prepared and samples sterilized at various temperatures and for various lengths of time until a series of ten samples were obtained ranging in color from that of non-sterilized evaporated milk to a deep brown.

Permanent standards were then prepared from $CaCO_3$ (precipitated chalk) suspensions to which were added varying amounts

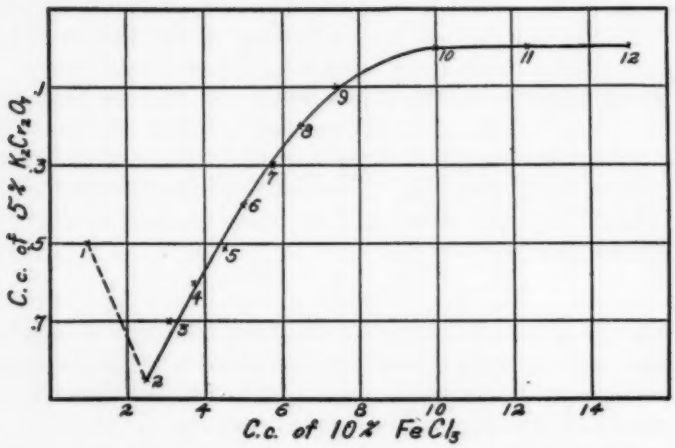

FIG. 2. AMOUNTS OF $K_2Cr_2O_7$ AND $FeCl_3$ SOLUTIONS ADDED TO 200 CC. OF A $CaCO_3$ SUSPENSION (1:5) TO PREPARE EACH COLOR STANDARD

Standard no. 1—to match unsterilized milks. Standards nos. 2 to 12—to match sterilized milks.

of solutions of 10 per cent $FeCl_3 \cdot 6H_2O$ and 5 per cent $K_2Cr_2O_7$ until these suspensions seemed to match in color the various milk samples previously prepared. These two sets of standards were then subjected to comparison by direct color measurements by the Munsell system. It was early noted that the artificial standards darkened slightly upon aging. Measurements from time to time indicated that equilibrium was reached at the end of approximately one month.

New standards of the $CaCO_3$ suspensions were prepared and adjusted in color with the $FeCl_3$ and $K_2Cr_2O_7$ solutions until their readings at the end of a month's storage checked approximately those of the evaporated milks. Each of the standards was placed in a 250-cc. flat-faced clear glass bottle and sealed to prevent loss of moisture. In making comparisons similar bottles were used

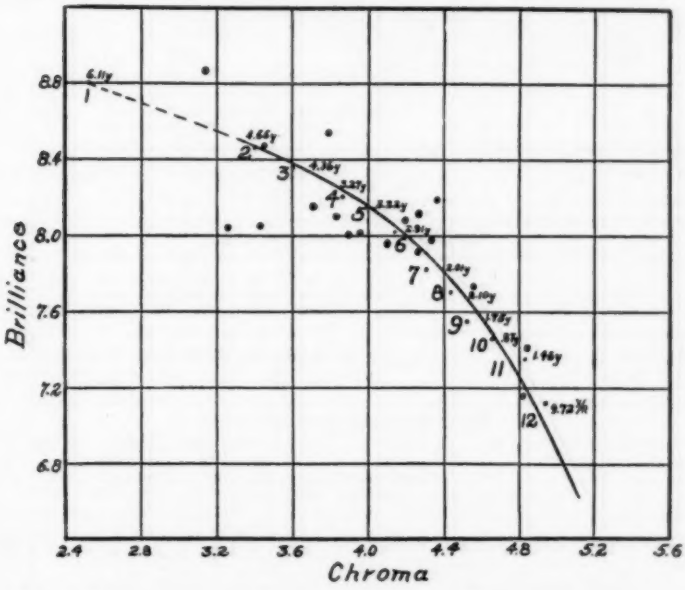

FIG. 3. MEASUREMENTS OF COLOR OF EVAPORATED MILKS AND ARTIFICIAL STANDARDS ACCORDING TO THE MUNSELL SYSTEM

● = commercial samples. ⊙ = experimental samples. • = artificial standards. The hue is inserted for each standard.

for the milk samples. A rack to hold the bottles with a wide slit opening to expose a flat surface of uniform size of each sample greatly facilitated comparisons. Comparisons are best made in diffused light from a north window.

In figure 2 are shown the amounts of solutions of 5 per cent $K_2Cr_2O_7$ and 10 per cent $FeCl_3 \cdot 6H_2O$ which were added to each

200 cc. of a 1:5$CaCO_3$ in water suspension to produce a standard. The addition of $FeCl_3$ solution imparts a brown coloration to the suspension while the addition of the $K_2Cr_2O_7$ imparts a yellow and slightly greenish hue.

TABLE 1

Effect of time and temperature of sterilization upon color and final pH of evaporated milk

Sterilization temperature 108°C.

	TIME							
	Check	5 minutes	10 minutes	15 minutes	20 minutes	25 minutes	30 minutes	35 minutes
pH	6.36	6.32	6.29	6.28	6.25	6.22	6.20	6.17
Color	1−	1+	1++	2−	2	2+	3	3+

Sterilization temperature 112°C.

	Check	5 minutes	10 minutes	15 minutes	20 minutes	25 minutes	30 minutes	35 minutes
pH	6.36	6.28	6.24	6.23	6.22	6.20	6.15	6.12
Color	1−	1+	2−	2	2+	3+	5−	6−

Sterilization temperature 116°C.

	Check	4 minutes	8 minutes	12 minutes	16 minutes	20 minutes	24 minutes	28 minutes	32 minutes
pH	6.36	6.28	6.22	6.19	6.14	6.10	6.07	6.03	6.02
Color	1−	1+	2−	2	3	4	5	6	7+

Sterilization temperature 120°C.

	Check	3 minutes	6 minutes	9 minutes	12 minutes	15 minutes	18 minutes	21 minutes	24 minutes	27 minutes	30 minutes
pH	6.36	6.23	6.20	6.18	6.15	6.12	6.06	6.02	6.00	5.95	5.90
Color	1−	1+	2−	3−	4−	5	6	7	8	9	10−

In figure 3 are given the readings upon the milks and the artificial standards prepared in terms of the Munsell notations. Brilliance is plotted against chroma, while the variations in hue for each standard are inserted in the figure.

For the particular milk samples first used the artificial standards prepared were a good imitation. Other milks prepared subsequently indicated considerable variation in readings in the region of lower chroma values, and it seems probable that standards Nos. 1, 2, and 3 (especially 1 and 2) should be of greater brilliancy to check the milk samples more perfectly. For practical purposes, however, these standards are seldom used since practically every evaporated milk sterilized sufficiently to keep well corre-

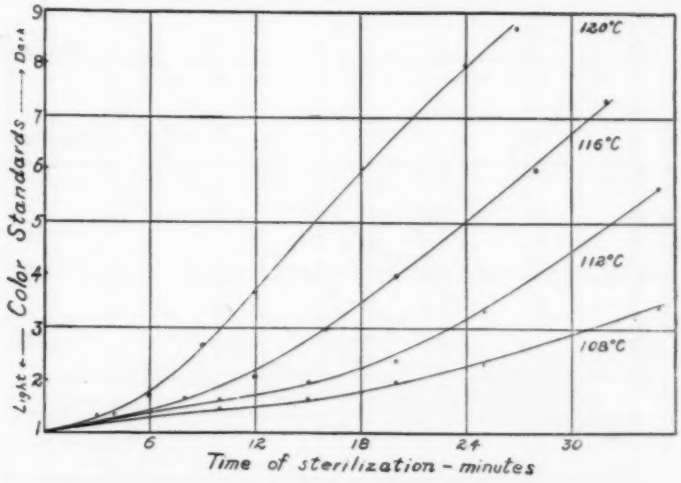

FIG. 4. EFFECT OF TIME AND TEMPERATURE OF STERILIZATION UPON THE COLOR OF EVAPORATED MILKS

sponds in color to some standard of greater color depth, an average commercial milk having a color matching standards No. 4 or 5.

The twelve standards selected for use in this work parallel the shades of evaporated milk from unsterilized (No. 1) to heavily sterilized samples (No. 12). In the data given the deviations from any standard are reported as + or − that number, the degree of color difference between each number in the following sequence being considered of equal value: 1, 1+, 2−, 2+, etc.

Factors influencing color variations of evaporated milks

Time and temperature of sterilization. The time and temperature of sterilization are the most important factors influencing the color of the evaporated product. An increase of exposure to heat is accompanied not only by an increase in color but also by a corresponding lowering in the final pH of the product. These

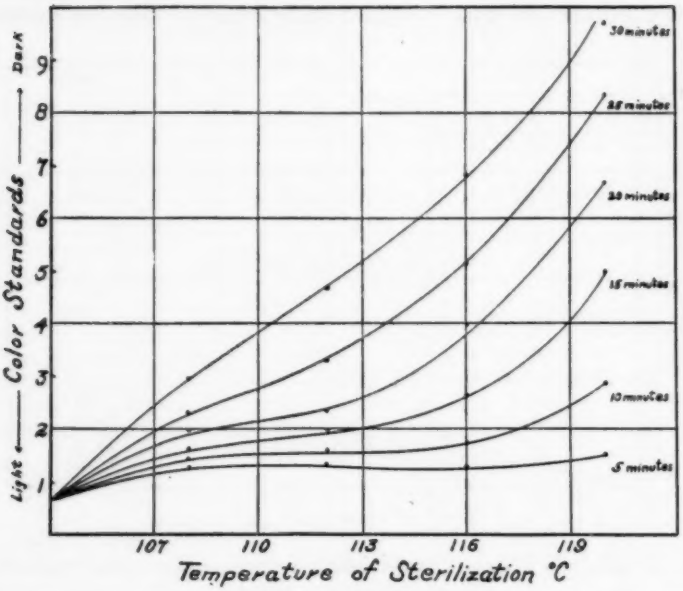

FIG. 5. EFFECT OF TIME AND TEMPERATURE OF STERILIZATION UPON THE COLOR OF EVAPORATED MILKS

relationships are shown by representative data, upon the same milk, given in table 1. Data from this table showing the effect of time and temperature of sterilization on color are plotted in figure 4. This figure indicates that the production of color is not a direct function of time but that the reaction is of a catalytic nature. With respect to temperature, for any chosen time of sterilization, the relationship is of the same nature, as shown in figure 5.

Although there is a continuous change in the H-ion concentration of the sterilized milks, as shown in table 1, their color is not a direct function of their pH. This is illustrated in figure 6.

Time of forewarming. The length of time of forewarming increases the exposure to heat and hence should cause the milk to reach the true logarithmic phase of color production more rapidly when it is sterilized. Table 2 indicates, however, that within the

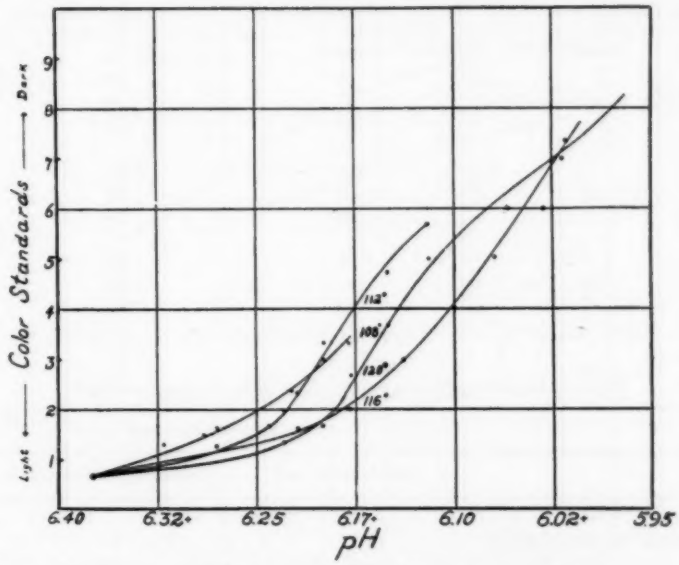

FIG. 6. RELATIONSHIP OF COLOR AND pH OF MILKS DURING STERILIZATION AT DIFFERENT TEMPERATURES

time of forewarming usually applied there is no great change in the color of the resulting product. For extremely long periods (thirty minutes) the color is greatly affected.

Homogenization. Homogenization breaks up the larger fat globules and hence diminishes the color of the product. Some of the results obtained are given in table 3.

Addition of neutralizers. At times the addition of neutralizers to evaporated milk influences the color of the product when ster-

ilized. Additions of small amounts of phosphates, citrates, and lime water failed to produce any change in color upon the milks used. However, a darkening of color was observed upon the addition just previous to sterilization of various amounts of a solution of 10 per cent sodium bicarbonate. A gradual deepening

TABLE 2

Effect of time of forewarming upon the color of the finished product

TIME OF STERILIZATION AT 118°C.	TIME OF FOREWARMING AT 95°C.	COLOR AFTER STERILIZATION
minutes	*minutes*	
Check—no sterilization	1	1+
	15	1+
	30	1++
12	1	5
	15	5+
	30	7−
18	1	8+
	15	9+
	30	11−

TABLE 3

Effect of homogenization upon the color of the finished product

TIME OF STERILIZATION AT 118°C.	COLOR AFTER STERILIZATION	
	Not homogenized	Homogenized at 3000 pounds
minutes		
Check—no sterilization	1	1−
5	2	2−
10	3+	3
15	6	5
20	8	6
25	10+	8

of color was observed in every case where this salt was used as a neutralizer. However, when the added $NaHCO_3$ was neutralized with lactic acid to the pH of the milk before sterilization, there was no measureable darkening in color of the final product as shown in figure 7. Data for a number of different milks to which $NaHCO_3$ was added are also plotted in figure 7.

Storage. Storage of sterilized evaporated milks results in an increase in their color. Samples of normal homogenized evaporated milks sterilized at 118° for 13 minutes were prepared and stored at the following temperatures: 5°, 10°, 20°, 30°, and 37°C. These samples were compared with the color standards at various intervals up to three months' time. No change in color

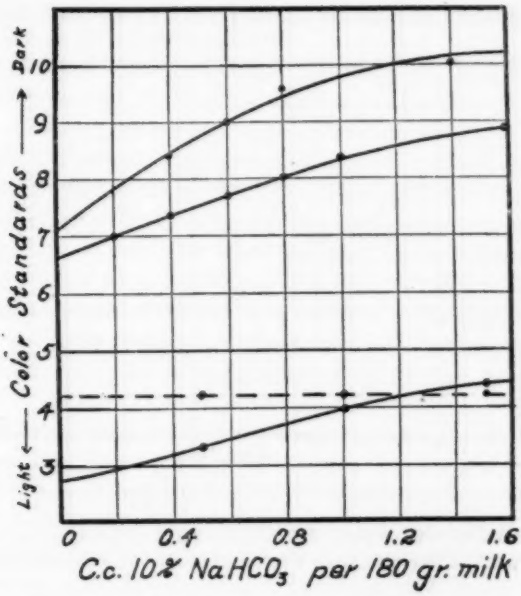

FIG. 7. EFFECT OF $NaHCO_3$ UPON COLOR OF EVAPORATED MILKS

——— $NaHCO_3$ added. ---------- Sodium lactate, at same pH as milk added. Constant time and temperature of sterilization for each series.

was noted in the milks stored at 5°C. Progressive darkening with increase in temperature and time of storage was noted in the other milks. After the sixth week of storage those milks held at 30° and 37°C. would no longer match in color any of the artificial standards. A color change of a different nature from that observed during sterilization was taking place.

Actual color measurements made with the Munsell equipment upon milks stored for three months at various temperatures showed this color change which occurred during storage to be one of chroma only, the brilliance and hue changing but slightly. This change in chroma resulted in a milk having a yellowish-green appearance. In figure 8 are plotted data representing the color change of a normal evaporated milk during sterilization at 118°C. and after storage for 3 months at 10°, 20°, 30°, and 37°C.

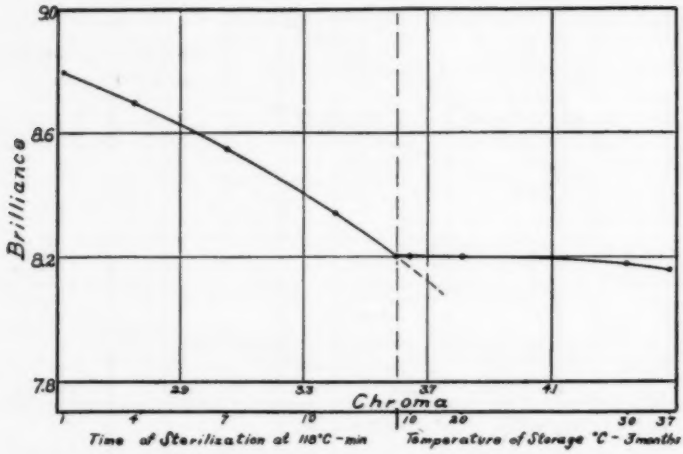

FIG. 8. CHANGES IN THE COLOR OF EVAPORATED MILK AS EFFECTED BY STERILIZATION AND THREE MONTH'S STORAGE AT VARIOUS TEMPERATURES

DISCUSSION

The use of $CaCO_3$ as a base, with $FeCl_3$ and $K_2Cr_2O_7$ to impart color, makes a permanent and satisfactory material for evaporated milk color standards. To obtain standards of uniform differences and a numerical expression for each color, the standards were read and recorded according to the Munsell color system. These standards may be reproduced with a limit of error of one-half to one degree of difference, a degree here being considered the color difference between any two consecutive standards. Difficulty was encountered in obtaining standard

the color of which conformed exactly to the color shades of milks from various sources. With a constant milk supply, the pigments will not vary enough to cause difficulty when the exact shade has been obtained. The $FeCl_3$ increases the red-brown color while the $K_2Cr_2O_7$ imparts a green-yellow color. By proper blending of these two chemicals in the white base any desired evaporated milk color may be duplicated. Three to four degrees, however, should be allowed for darkening during the first month before equilibrium is reached.

The time-temperature relationship during sterilization is the most important factor affecting the color of evaporated milk. Since this relationship closely approximates a constant in industrial practice, however, it should not cause a lack of color uniformity in a commercial product. Considering the above relationships and other effects of heat upon color, chiefly that of time of forewarming, and of the effect of heat upon concentration, certain conclusions seem justified. The darkening of color caused by heat in evaporated milk seems to be of a catalytic nature, dependent probably upon the production of substances in the solution which increase the susceptibility of the lactose to caramelization.

The only important neutralizer which appears to affect appreciably the color of the product is sodium bicarbonate. The increase of color due to the addition of this salt was not found to be uniform for all milks to which it was added. Where no change in pH was brought about by addition of the neutralizer, as when salt was added after it had been neutralized with lactic acid, no darkening of the color of the finished product was observed. In those cases in which color darkening was observed, the addition of the neutralizer did not bring about maximum stability in the darkest colored samples. It appears, therefore, that where neutralization is properly conducted to obtain maximum stability, minimum darkening of color will result, but where overneutralization is practiced the color will be unduly increased. In no case, however, will the color of neutralized milk be as light as that of the milk to which no salt has been added.

The complete color change of an evaporated milk as presented

in figure 8 shows it to be of a catalytic nature during sterilization, while the change during storage, which is proportional to time and temperature, is one of chroma only, giving the milk a yellowish-green color. The fact that the color change during storage does not continue along the sterilization curve seems to indicate that factors other than caramelization of the lactose affect the color of the stored milk.

SUMMARY

Permanent color standards have been developed, expressed in numerical terms according to the Munsell system.

Color changes caused by variation in the manufacturing process of evaporated milk have been studied. The effect of heat, whether encountered during forewarming, sterilization, or storage, was found to produce important changes in color. An increase in heat produced an increase in color, the change being of a catalytic nature. Increase in heat during sterilization deepened color by affecting brilliance, chroma, and hue; while increase in time and temperature of storage deepened color through increase in chroma only. The use of sodium bicarbonate as a neutralizer darkened the color of evaporated milk in proportion to the amount used.

ACKNOWLEDGMENT

The authors are indebted, for use of their Munsell color equipment, to the Bureau of Agricultural Economics, where the method of applying the Munsell system, including formulas and apparatus, has been developed by Miss Dorothy Nickerson, color technologist, in connection with the research program of the Division of Cotton Marketing and the standardization work of the Hay, Feed, and Seed Division.

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A COMPARISON OF THE VOLUMETRIC AND GRAVIMETRIC METHODS FOR BACTERIOLOGICAL EXAMINATION OF ICE CREAM*

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INTRODUCTION

A bacteriological analysis of ice cream presents certain difficulties which hinder the procurement of a fair sample. Obviously, the air incorporated in the product must be completely expelled if uniform results from volumetric (1- or 10-cc.) samples are to be expected. On the other hand, if the analysis is made on a gravimetric basis (10-gram), the sample need only be melted sufficiently to enable thorough shaking. Variation in the viscosity of different ice cream mixes and the use of glassware that is not chemically clean results in more or less of the mix adhering to the pipette. With a volumetric sample this may offer a source of considerable variation, whereas with a gravimetric portion it does not matter how much mix adheres to the pipette or how much air is retained in the product. There has been some question in the minds of those who make bacterial determinations on ice cream as to whether the advantages of the gravimetric sample justify the extra time and work involved. The use of the gravimetric method necessitates careful counter-balancing of the dilution blank, followed by carefully weighing 10 grams of the melted ice cream. The two operations with the balances are both time consuming and tedious. Bolling and his associates (4) found that the gravimetric method required 77 per cent more time than the volumetric procedure and concluded that, "equally accurate counts could be obtained by either method." The extra labor involved in the gravimetric method would be justified only if the results were less variable than would be obtain-

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able from the more simple and less time consuming volumetric method. The following experiments were designed primarily to ascertain the comparative degree of variability of the gravimetric and volumetric methods.

PLAN OF THE EXPERIMENT

After preliminary plating of a sample of ice cream to determine the desired dilution, about 250 cc. of the frozen product were

TABLE 1

A comparison of the results obtained with gravimetric and volumetric samples in the bacteriological analysis of ice cream

EXPERIMENT NUMBER	DILUTION	NUMBER OF PLATES	AVERAGE NUMBER OF COLONIES PER PLATE			PROBABLE ERROR OF SINGLE PLATES EXPRESSED IN COLONIES PER PLATE			NUMBER OF PLATES NEEDED TO INSURE (30 TO 1 CHANCE) THAT THE DIFFERENCE BETWEEN THE MEANS IS SIGNIFICANT—COMPARISON OF					
									10-gram and		10-cc. and		1-cc. and	
			10-gram sample	10-cc. sample	1-cc. sample	10-gram sample	10-cc. sample	1-cc. sample	10-cc. sample	1-cc. sample	10-gram sample	1-cc. sample	10-gram sample	10-cc. sample
1	1:1,000	150	32	33	36	4.65	4.99	4.78	221	14	255	28	15	26
2	1:10,000	148	52	54	56	4.71	5.43	5.90	57	14	75	75	22	89
3	1:100	144	150	157	130	25.48	19.78	33.51	135	17	82	5	29	16
4	1:1,000	118	169	192	180	17.76	16.69	23.85	6	27	5	20	48	40
5	1:500	150	194	180	187	28.23	12.70	14.47	42	166	8	34	44	44
6	1:100	150	226	231	248	20.26	22.62	24.67	168	9	209	18	13	22
7	1:10,000	149	269	231	205	45.15	26.04	36.05	14	5	5	10	3	20
8	1:1,000	150	227	266	305	29.05	38.91	23.47	71	11	128	10	7	4
9	1:1,000	150	465	538	566	101.42	70.41	74.76	20	10	10	65	6	73
10	1:1,000	149	2,270	2,175	2,339	185.59	184.81	205.85	39	74	39	13	91	16
11	1:500	150	120	124	146	27.00	24.63	32.15	467	11	388	13	16	22
Average.....			384	310	400				112	32	109	26	26	33

melted in a water bath at 45°C. until as much of the air had been expelled as possible. The time required for melting did not exceed fifteen minutes in any case.

Five sets of dilutions were made from 10-gram samples and 10 plates were poured from the final dilution of each set, thereby making 50 plates on a gravimetric basis. Similarly, 5 sets of dilutions each were prepared from 10-cc. and 1-cc. volumetric samples

and 50 plates poured from the final dilutions in each case. In no case did the elapsed time between weighing or measuring the sample and pouring of the agar exceed fifteen minutes. Occasionally it was necessary to eliminate a contaminated plate, and in a few instances to eliminate a whole series of 10 plates, due to a contaminated dilution blank. In most instances, however, the data are based on the entire set of 150 plates.

The experiment as described was followed on 10 samples of ice cream. Another procedure which was followed for an eleventh sample will be described when the data are presented.

The methods used for preparation of media, sampling, weighing, plating, and incubation were those set forth in the report of the American Dairy Science Committee on Bacteriological Methods for Examining Ice Cream (1).

In selecting the samples of ice cream for analysis and in estimating the desired dilution, an attempt was made to obtain sets of 150 plates with numbers of colonies ranging from a few colonies per plate (about 30) to an excessive number.

By statistical treatment of the data collected, it is possible to show the comparative variation of the three methods of sampling. It is also possible to determine whether or not the differences in the results obtained by the three methods are significant.

RESULTS

A study of the average counts reported in the table shows that in some experiments the 1 cc. volumetric method of sampling yielded the highest average count, whereas in other samples one of the other methods gave the highest counts. Space does not permit a detailed discussion of the figures in this table, but only a brief examination of the data will convince the reader that neither method consistently gave the highest or lowest results.

Mudge and Lawler (2) have questioned the applicability of statistical methods to plate counting. Their criticism is based on the observation of factors other than chance which affect the results when a large number of plates are prepared from a single dilution blank. Their results show that in making 75 plates from the same dilution blank, the last 35 of the series give mate-

rially higher counts than the first 40 plates, even though the dilution blanks are chilled. More recently (3) these investigators have attributed this phenomenon to the lack of buffering effect in distilled water dilution blanks, and have found that it could be prevented by using buffered solutions for dilution.

Obviously, an erroneous conception of the degree of variability of the plate count would be drawn if data included in the computations are affected by this phenomenon. Although the work reported in the present paper was completed before Mudge and Lawler's report of the use of buffered dilution blanks, an effort was made in these experiments to eliminate the error introduced by this phenomenon.

The results obtained by Mudge and Lawler indicate that some force is at work in the dilution blank which causes the bacterial clumps to disintegrate. Whatever the cause of this phenomenon may be, its appearance is more or less sharply defined after about 40 plates have been poured. It is not known whether the clumps suddenly disintegrate after about 40 plates have been poured or whether the process begins immediately after making the dilution and only attains significant magnitude after a given time interval. If the former be the case, statistical analysis of the results of more than about 40 plates per dilution blank would be unjustified and misleading. On the other hand, if the separation of clumps in the unbuffered blanks is a gradual process, it is one of the factors of normal variation of the plate method up to fifteen minutes after the preparation of the dilution, and should be included in a statistical analysis. In either event, it is evident from the work of Mudge and Lawler, that a statistical analysis of plate counting should not be based on more than 30 to 35 plates from single unbuffered dilution blanks. On the other hand, unless buffered dilution blanks were commonly employed in laboratory procedure, it would be equally misleading to judge the error of plate counting from data based on series of 75 or 100 plates made from buffered dilutions.

In view of the facts that the unbuffered blanks, as employed in this work, are commonly used for dilution, that only 10 plates were poured from each blank, and that the time interval between

making and plating the dilution did not exceed fifteen minutes, it is believed that the variations in these data are of practical significance. The statistical constants are likewise believed at least to be representative of the expected limits of variation which will be encountered in bacterial analyses by the plate method. The statistical values given are not directly applicable to the work of another laboratory and, very likely, not applicable to previous or subsequent work of this laboratory. At best, these values give only some tangible evidence of the general range of variation of the plate method of analysis.

In the first experiment, it may be observed that the probable error of single plates made from the 1-cc. samples was the lowest; in the second experiment, the 10-gram samples gave the least variation; and in the third experiment, the 10-cc. samples showed less variation than the others. If the remainder of the figures in these three columns are examined it will be observed that neither method of sampling is significantly superior to the others. With one sample of ice cream one method may yield slightly less varying results, but with the next sample the degree of variation previously observed may be completely reversed.

In order to further illustrate that there is little on which to base a choice of these methods of sampling, in the last six columns of the table are presented the number of plates required to show a significant difference in the results obtained by various combinations of methods. The figures for experiment 5 will be used for explanation. It will be noted that the averages of 50 plates each from 10-gram, 10-cc. and 1-cc. samples were 194, 180, and 187 colonies respectively. Since these values were obtained from averaging a larger number of plates than would ever be made in routine analysis, it will be arbitrarily assumed, for sake of the subsequent explanation, that for this ice cream 194, 180 and 187 are the "true values" for these respective methods of sampling. On the basis of this assumption, therefore, the difference of 14 colonies (194 − 180) represents the difference in the counts obtained with 10-gram, and 10-cc. samples, and, likewise, 7 colonies represents the difference when the 10-gram and 1-cc., and the 10-cc. and 1-cc. samples are compared. The figures in the table

indicate that in order to demonstrate conclusively (30 to 1 chance) that the difference between the 10-gram and 10-cc. samples is real, it would require 42 plates by the gravimetric method and 8 plates by the 10-cc. volumetric method. Simliarly, in order to demonstrate a significant difference between results obtained with the 10-gram and 1-cc. volumetric methods it would require 166 plates by the former and 44 by the latter. Likewise, it may be seen that it would take 34 plates by the 10-cc. volumetric method and 44 by the 1-cc. method to demonstrate the significance of the difference in the results of these two methods of sampling.

In determining the number of plates required to demonstrate the significance of the difference between the averages of 50 plates the following formula was used,

$$N = \left(\frac{c \cdot p}{d}\right)^2,$$

where N = number of plates
c = coefficient of odds (3.2 used in this case which gives 30 to 1 chance)
p = probable error of a single plate
d = difference between the means

When comparing two methods, e.g. the 10-gram and 10-cc. samples, it was necessary to calculate the requisite number of plates for each method of sampling since the degree of variation (p) was not the same in each case.

A study of this table indicates that in order to demonstrate a preference of one method of sampling over another it would require far more plates than are ordinarily used in routine analytical work. The required number varies from 3 to 467 plates, and in only 11 of the 66 comparisons was the number of plates less than 10.

As previously stated, a different procedure was followed in the analysis of the eleventh sample of ice cream (experiment 11). In this experiment 25 separate samples from the same can of ice cream were taken in 25 sterile sample jars. All of the samples were melted simultaneously in the same water bath and for the same length of time. After melting all were placed in iced-water until used. From each sample of the melted ice cream a 10-gram,

a 10-cc., and a 1-cc. portion were taken for subsequent dilution. From the final dilution duplicate plates only were poured. Thus it may be seen that there were 25 separate weighings of 10-gram portions from 25 separate sample jars of the same ice cream, and that there were duplicate plates for each determination. Similarly, there were 25 separate determinations (50 plates) each on a 10-cc. and a 1-cc. basis. It was thought that in some respects the results from this procedure would more closely approximate the error of the method as used in practice than the procedure followed in the other experiments.

It may be noted in experiment 11 that the plates from the gravimetric samples averaged 120 colonies per plate with a probable error for a single plate of 27 colonies; the 10-cc. volumetric samples averaged 124 colonies per plate with a probable error for a single plate of 24.63 colonies; and the plates from the 1-cc. samples averaged 146 colonies with a probable error for a single plate of 32.15 colonies. The number of plates necessary to show a preference of one method over another varied from 11 to 467, again emphasizing that the results of routine analysis in which duplicate or triplicate plates are used would not yield significantly different results regardless of the method employed.

DISCUSSION

It is impractical to make fine measurements with a crude measuring stick. The error inherent in the plate method obliterates the slight advantage which one type of sample may have over another. It undoubtedly is true that a 10-cc. sample is more representative than a 1-cc. sample. Nevertheless, in these experiments, the counting of approximately 500 plates by each of the methods indicates that the differences in results obtained are so small as to require large numbers of plates to substantiate a preference. Similarly, any theoretical advantage which may accrue from the use of a 10-gram gravimetric sample necessitates in most instances excessive numbers of plates to corroborate. The data indicate quite clearly that the errors introduced by the various methods of sampling are overshadowed by the error of the plating method.

After studying these results one is forced to conclude that it makes little difference whether an ice cream analysis is based on a 10-gram, 10-cc. or 1-cc. sample. Although it is perfectly logical to assume that a 10-gram sample eliminates certain errors, and that a 10-cc. sample is more representative than a 1-cc. sample, nevertheless, the normal variation tends to obliterate the superiority which any one method may possess. If, as indicated in these data, either of the volumetric samples yields results as reliable as the more tedious and time consuming gravimetric procedure, there is little to justify the use of the latter method. On the other hand, a 10-gram sample certainly would be advisable when an occasional sample of ice cream is found to be frothy on melting and does not give up its incorporated air. In choosing between a 10-cc. and a 1-cc. sample, it seems logical to recommend the 10-cc. sample because it is more representative, however, it would be difficult to substantiate the preference if the plate method of analysis were employed.

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SOME FACTORS INFLUENCING THE VOLUME OF FOAM ON MILK*

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The appearance of foam on milk and some of its products is a commonly observed phenomenon. In some instances, as in the whipping of cream or in the manufacture of ice cream, a thick, stable foam is desired. In other instances, as in the filling of vats, cans, bottles, or other containers, or in the operation of machines, such as clarifiers, separators, pasteurizers, etc., the formation of foam may present a serious technical problem. The overflow of foam when vessels are to be filled may result in the loss of a considerable quantity of milk solids.

It is known to bacteriologists that in the commercial pasteurization of milk, the destruction of bacteria in the foam layer is far less complete than in the remainder of the milk. Whittaker, Archibald, Leete, and Miller (9) have shown that the temperature of the foam on the surface of a vat of milk during the pasteurization process may be much below that which is necessary to insure complete destruction of the pathogenic bacteria sometimes found in milk. They have shown, also, that under certain conditions which are frequently found in dairy plants, the bacterial content of the foam layer may actually increase during pasteurization.

Rahn (3) claims that milk contains a special foam compound (a protein), which is neither casein nor albumin, but which probably is the protein that surrounds the fat globules.

Measurements reported by Siedel (8) indicate that whole milk churned for about forty-five minutes at a low temperature has lost its ability to produce foam, on the skim milk obtained from it subsequently by centrifugal separation. This author also

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reported data showing that the loss of foaming ability was not due to the low temperature during the churning but rather to the mechanical agitation of the milk.

The associates of Rogers (7) report that the tendency for milk to foam is at a minimum between the temperatures of 20° and 30°C. (68° and 86°F.). Below this range the foaming tendency increases with decreases in temperature; above this range, there is a rapid increase in the foaming tendency with increases in temperature. These authors also report that pasteurization slightly reduces the tendency to foam at the various temperatures, and that the region of minimum foaming ability shifts to a higher temperature as the fat content of the milk increases.

Dahlberg and Hening (1) report an experiment in which a sample of pasteurized skim milk whipped at 4.4°C. attained a much larger volume than a similar sample of raw skim milk. Milk with 10 per cent of fat whipped to a much smaller volume than did skim milk.

Larson (2) in a preliminary report stated that foam production on milk took place in a vacuum to approximately the same degree as under atmospheric pressure.

The present investigation was planned to make a systematic study of the influence of certain factors on the foaming ability of cow's milk.

METHOD

Portions of the sample, each 250 ml. in amount, were measured with a graduated cylinder and transferred to 500-ml. graduated beakers. The different portions were tempered in the beakers in baths of water to the temperatures required. Each measured sample was then whipped for one minute in the beaker by an electrical stirring machine, such as is used commonly at soda fountains in the preparation of malted milks and other drinks. At the end of the minute the agitator was quickly withdrawn. Fifteen seconds was allowed for the surface of the foam to come to rest, after which the total apparent volume of the sample (including foam) was read quickly.

The apparent increase in volume of the sample was calculated

as a percentage of the original volume (250 ml.) and designated as the "percentage increase in volume due to foam."

Preliminary trials indicated that duplicate observations on the same sample could be expected to fall within ±5 ml. of the mean when small apparent increases in volume occurred, and within ±10 ml. of the mean when the larger increases were encountered. Usually duplicates agreed closer than these extremes. In some cases it was necessary to calibrate the beakers beyond the 500-ml. mark.

TABLE 1

Effect of temperature at time of whipping on foaming ability

TEMPERATURE	PERCENTAGE INCREASE IN VOLUME DUE TO FOAM		
	Whole milk	Skim milk	20 per cent cream
°F.			
35	88	130	49
40	73	122	40
50	30	84	36
60	11	28	26
70	8	18	20
80	8	17	15
90	16	21	8
100	29	26	31
110	32	29	35
120	31	28	37
130	30	27	37
140	28	25	35
150	26	24	31
160	25	24	29
170	23	23	27
180	22	21	25

EFFECT OF TEMPERATURE ON FOAMING ABILITY

Temperature of the sample at the time of whipping

The foaming ability of each of 8 lots of commercially pasteurized milk, 6 lots of commercially pasteurized skim milk, and 2 lots of commercially pasteurized 20 per cent cream was determined at temperatures from 35° to 180°F. The averages of the results are shown in table 1.

The data indicate that in all 3 products, the greatest amount of

foam was produced at the lowest temperature of measurement. With increases in temperature, the volume of foam decreased until a minimum was reached. This minimum was reached at 70° to 80°F. with whole milk, 80°F. with skim milk, and 90°F. with 20 per cent cream.

Further increases in the temperature of the samples resulted in increasing the foaming ability, until a second maximum point was reached. This second maximum point was reached at 110°F. with whole milk and skim milk, and at 120° to 130°F. with 20 per cent cream.

The results show, also, that at low temperatures skim milk foamed most, whole milk next, and 20 per cent cream least. At the higher temperatures of measurement, the reverse relationship existed. If the data were plotted, it would appear from the graphs that at about 96° to 97°F. the volumes of foam on the 3 products should be almost identical.

Previous heating (pasteurization)

Separate portions of the same lots of fresh whole milk (4 lots) and of fresh skim milk (3 lots) were heated for thirty minutes at 140°F. Separate portions of other lots of fresh whole milk (3 lots) and of fresh skim milk (3 lots) were heated for thirty minutes at 180°F. The heated portions were promptly cooled to below 50°F., and distilled water added to replace that lost by evaporation. All of the samples, together with unheated controls, were stored for two or three days in a refrigerator at 40°F.

Measurements of the foaming ability were then made in duplicate, at temperatures of 40°, 80°, 140°, and 180°F. The averages of the results are shown in table 2.

The results indicate that heating milk or skim milk for thirty minutes at 140°F. had no significant effect on the foaming ability. When the heating was done at 180°F. for thirty minutes, there was a slight but regular decrease in the foaming ability.

Freezing

Samples of pasteurized whole milk, skim milk, and 20 per cent cream were kept at a temperature of about −10°F. for two days.

The frozen samples then were kept at room temperature for a day to melt, and thereafter stored for two or three days at about 40°F. Control samples were kept continuously at 40°F.

Duplicate measurements of the foaming ability were made at 40°, 80°, and 140°F. The results are shown in table 3.

TABLE 2

Effect of previous heating on foaming ability

DESCRIPTION OF SAMPLE	PERCENTAGE INCREASE IN VOLUME DUE TO FOAM			
	40°F.	80°F.	140°F.	180°F.
Raw whole milk, control (4 lots).....	94	7	26	20
Same, but heated, 140°F.—30 minutes...	89	8	27	20
Raw skim milk, control (3 lots).....	133	26	28	27
Same, but heated, 140°F.—30 minutes...	133	28	27	23
Raw whole milk, control (3 lots).....	95	7	27	*
Same, but heated, 180°F.—30 minutes...	78	6	25	*
Raw skim milk, control (3 lots).....	117	27	29	*
Same, but heated, 180°F.—30 minutes...	112	22	26	*

* Measurements omitted.

TABLE 3

Effect of freezing on foaming ability

DESCRIPTION OF SAMPLE	PERCENTAGE INCREASE IN VOLUME DUE TO FOAM		
	40°F.	80°F.	140°F.
Whole milk, control.....	95	8	27
Whole milk, frozen.....	89	7	27
Skim milk, control.....	136	31	29
Skim milk, frozen.....	136	30	30
20 per cent cream, control.....	54	11	38
20 per cent cream, frozen.....	38	16	38 ("oiled off")

The results indicate that freezing decreases slightly the foaming ability of whole milk when the measurements are made at 40°F., but not at 80° or 140°F. The results with 20 per cent cream indicate a considerable decrease when the measurements are made at 40°F., an increase at 80°F., and no change at 140°F. There appears to be no effect on the foaming ability of skim milk as the result of freezing.

It seems that the effect of freezing on the foaming ability may be due to the physical condition of the milk fat. Reid (6) has shown that in freezing the globules of fat in milk become much distorted and grouped. Apparently, the distorted and grouped globules interfere mechanically with the normal tendency of the milk to foam. When the measurements were made at 80° or 140°F., at which temperatures the fat globules were softened or melted, the decrease in foaming ability was not noted. Skim milk, containing but a trace of fat, showed no change due to freezing.

EFFECT OF AGE AND OF STORAGE TEMPERATURE ON FOAMING ABILITY

In a preliminary experiment with 6 lots of whole milk from various sources, it was found that when the milk was passed through a centrifugal separator while quite fresh, the foaming ability of its skim milk was much greater than after the milk had been held for from twenty to seventy-two hours in a refrigerator. The results suggested that the foaming ability of milk might be expected to decrease with age.

Milk was obtained at milking time and poured into a warmed insulated can. One portion was at once poured into warmed milk bottles and stored at 98°F. for three to four hours. Another portion was cooled quickly in ice water to 40°F., and stored at that temperature for three to four hours. The foaming ability of the original, fresh milk was determined as a control. Duplicate measurements were made at 40°, 80°, and 140°F. The foregoing operations were completed within an hour after the collection of the milk at the barn was begun.

The foaming ability of the milk stored at 98°F. and of that stored at 40°F. were determined as above. The experiment was repeated with a second lot of milk. The averages of the results are shown in table 4.

These results indicate that the temperature at which milk is held is significant in determining the effects of age on the foaming ability of the milk. The measurements made after the milk had been held for three to four hours at 98°F. were practically identical with those made when the milk was freshly drawn. In

the freshly drawn milk, and in the milk held at 98°F., the measurements made at 80°F. and at 140°F. were approximately the same. In the preceding experiments in this study (all with previously cooled milk), the foaming ability of whole milk at 80°F. was found to be much less than at 140°F.

The milk which had been held at 40°F. for three to four hours showed a slight decrease in its foaming ability as determined at 40°F., and a great decrease as determined at 80°F. All of the samples showed the same foaming ability when the measurements were made at 140°F.

Apparently, the solidification and clumping of the fat globules, which occurs when milk is held cold, influences the foaming ability of the milk. In the sample stored cold and then tempered to

TABLE 4

Effect of age and of storage temperature on foaming ability

TREATMENT OF SAMPLE	PERCENTAGE INCREASE IN VOLUME DUE TO FOAM		
	40°F.	80°F.	140°F.
Fresh, control	98	24	25
At 98°F. for 3–4 hours	100	26	25
At 40°F. for 3–4 hours	86	8	25

80°F. the fat globules continued in the solid state, but certainly became somewhat softened. In the freshly drawn sample, and in the sample held at 98°F. when tempered to 80°F., the fat globules undoubtedly continued practically in the liquid state. In all of the samples, when tempered to 140°F., the fat globules were in the liquid condition.

EFFECT OF PREVIOUS AGITATION ON FOAMING ABILITY

Four different lots of raw whole milk, 3 of skim milk, and 2 of 20 per cent cream were each divided into 2 portions. About 3 or 4 gallons of one portion in each case was whipped for one hour in a motor driven 50-quart ice cream freezer cooled by brine. The temperature of the sample in the machine was kept between freezing and 40°F.

The second portion in each case was heated to 145°F., and distilled water added to replace that lost by evaporation. About 3 or 4 gallons of the heated sample in each case was whipped for one hour in the ice cream freezer. The machine had been heated previously so that the temperature of the product remained above 120°F. during the whipping. A sample of the heated, unwhipped portion was kept at practically the same temperature as long as the whipping continued. After the whipping, all of the heated samples were cooled promptly in ice water.

TABLE 5

Effect of previous agitation on foaming ability

TREATMENT OF SAMPLE	PERCENTAGE INCREASE IN VOLUME DUE TO FOAM								
	Whole milk			Skim milk			20 per cent cream		
	40°F.	80°F.	140°F.	40°F.	80°F.	140°F.	40°F.	80°F.	140°F.
Control (a)	87	7	23	136	19	28	44	11	33
Whipped at 40°F. or under (b)	31	4	15	128	14	23	*	*	*
Heated to 120°F. or over (c)	92	8	24	137	18	28	42	11	32
Whipped at 120°F. or over (d)	128	16	24	140	30	30	65	12	28
Decrease due to whipping cold (a–b)	56	3	8	8	5	5	—	—	—
Decrease due to heating and whipping warm (a–d)	−41	−9	−1	−4	−11	−2	−21	−1	5
Decrease due alone to whipping warm (c–d)	−36	−8	0	−3	−12	−2	−23	−1	4

* Badly churned; no measurements.

All of the samples and controls were stored for a day in a refrigerator at 40°F. Measurements of the foaming ability were made in duplicate at 40°, 80°, and 140°F. The averages of the results are shown in table 5.

The results indicate that the foaming ability of whole milk or skim milk was diminished by agitation while cold. When whole milk, skim milk, and 20 per cent cream were whipped at a temperature well above the melting point of milk fat, and subsequently cooled, the foaming ability generally was increased (negatively decreased).

Rahn (3) and Rahn and Sharp (4), citing Siedel's (8) data, claim that the formation of foam is an irreversible process. They claim that the foam substance solidifies in the surface film (foam) according to the phenomenon described by Ramsden (5), and then is no longer capable of producing foam. Previous agitation, which produces foam, then should decrease the subsequent foaming ability of the milk. The results obtained with the lots which were agitated while cold harmonize with this view. When the products were agitated while warm the data obtained gave no evidence that the foam substance was inactivated.

TABLE 6

Effect of homogenization on foaming ability

TEMPERATURE OF MEASUREMENT	PERCENTAGE INCREASE IN VOLUME DUE TO FOAM			
	Control heated only	Homogenized at 3000 pounds—first valve only	2000 pounds, first valve; 1000 pounds, second valve	3000 pounds, first valve; 1000 pounds, second valve
	Whole milk			
°F.				
40	91	112	112	109
80	7	20	18	19
140	29	24	25	23
	Skim milk			
40	131	136	137	134
80	29	33	34	33
140	28	31	31	32

EFFECT OF HOMOGENIZATION ON FOAMING ABILITY

A lot each of raw whole milk and skim milk were heated to 145°F. A portion of each heated lot was kept as a control. Other portions of each were passed through a two-stage homogenizer, such as is used in commercial dairy plants. Various degrees of pressure were used, as indicated in the table. The various homogenized portions and the controls were cooled promptly in ice water and then stored overnight in a refrigerator at 40°F. After storage, the foaming ability of each was determined in duplicate at 40°, 80°, and 140°F. The results are shown in table 6.

The data indicate that homogenization increased considerably the foaming ability of whole milk as measured at 40° and 80°F. There was a decrease when the measurements were made at 140°F. In all cases, the homogenized skim milk showed a slightly increased foaming ability.

EFFECT OF COMPOSITION ON FOAMING ABILITY

Two lots of milk from individual Ayrshire cows and 2 from individual Holstein cows were obtained. All were analyzed for fat and total solids. The foaming ability of each lot was determined, after cooling and storing overnight in a refrigerator. The measurements were made in duplicate at 40°, 80°, and 140°F. The results are shown in table 7.

TABLE 7

Effect of composition on foaming ability of milk from individual cows

SOURCE OF SAMPLE	FAT	TOTAL SOLIDS	PERCENTAGE INCREASE IN VOLUME DUE TO FOAM		
			40°F.	80°F.	140°F.
	per cent	*per cent*			
Ayrshire Cow 1	4.6	14.28	85	8	28
Ayrshire Cow 2	5.2	14.88	94	15	33
Holstein Cow 1	3.4	13.09	98	11	28
Holstein Cow 2	3.7	12.20	125	9	25

The results fail to indicate any definite relationship between the fat and total solids content of milk and its foaming ability. Apparently, the foaming ability of milk from individual cows is dependent largely on factors which are characteristic for the animals.

To determine the effect of composition on the foaming ability when the influence of the individual characteristics of the milk was standardized, samples were prepared by mixing high testing cream, skim milk, and distilled water in varying proportions. The cream and skim milk were obtained by machine separation of high testing milk produced by a herd of Jersey and Guernsey cows. The cream and skim milk were cooled and at once analyzed for their content of fat and solids not fat. As promptly

as possible, the cream, skim milk, and distilled water were mixed in separate lots to yield the possible combinations of 0, 2, 4, or 6 per cent of fat, with 1, 5, or 9 per cent of solids not fat. The prepared samples were stored overnight in a refrigerator at 40°F.,

TABLE 8

Effect of composition on the foaming ability of various prepared samples

Part A

FAT	PERCENTAGE INCREASE IN VOLUME DUE TO FOAM								
	1 per cent solids not fat			5 per cent solids not fat			9 per cent solids not fat		
	40°F.	80°F.	140°F.	40°F.	80°F.	140°F.	40°F.	80°F.	140°F.
per cent									
0	59	26	14	138	26	21	136	29	28
2	25	4	9	100	7	19	115	10	27
4	19	4	11	76	6	23	99	8	27
6	16	5	10	66	5	22	88	7	31

Part B

COMPOSITION OF MIXTURE			PERCENTAGE INCREASE IN VOLUME DUE TO FOAM		
Total solids	Fat	Solids not fat	40°F.	80°F.	140°F.
per cent	*per cent*	*per cent*			
5	0	5	138	26	21
5	4	1	19	4	11
7	2	5	100	7	19
7	6	1	16	5	10
9	0	9	136	29	28
9	4	5	76	6	23
11	2	9	115	10	27
11	6	5	66	5	22

and the foaming ability of each determined. The measurements were made in duplicate at 40°, 80°, and 140°F.

The experiment was performed 3 times, using different original lots of milk. The averages of the results are shown in table 8.

The data indicate that when the influence of the individual characteristics of the milk was standardized, the fat generally

had a depressing influence on the foaming ability of the sample, while the solids not fat generally increased the foaming ability. This is best shown in Part B of the table. In each pair of samples, the one with the more fat had the lesser foaming ability.

EFFECT OF DEVELOPED ACIDITY ON FOAMING ABILITY

Different lots of sweet whole milk and skim milk were tempered to 72°F. and inoculated with small amounts of lactic dairy starter. The lots were each divided into several portions, which were incubated for varying lengths of time at room temperature.

TABLE 9

Effect of developed acidity on foaming ability

Whole milk

PERCENTAGE ACIDITY	PERCENTAGE INCREASE IN VOLUME DUE TO FOAM											
	0.16	0.18	0.19	0.20	0.27	0.31	0.38	0.46	0.58	0.71	0.76	0.80
°F.												
40	104	104	102	104	109	119	134	135	130	103	63	42
80	9	8	8	8	9	8	8	9	9	10	10	12
140	29	29	28	28	27*	25*	24*	†	†	†	†	†

Skim milk

	0.15	0.17	0.19	0.22	0.30	0.33	0.44	0.57	0.68	0.80	0.83
40	133	134	136	134	134	135	135	147	138	87	57
80	20	20	19	20	20	20	21	20	20	20	29
140	29	28	28	28	27	27	19*	†	†	†	†

* Slight curdling during tempering.

† Curd separated; no measurements.

They were then stored for about two days in a refrigerator at 40°F.

The foaming ability in each case was determined after storage, duplicate measurements being made at 40°, 80°, and 140°F. The titratable acidity, calculated as lactic acid, was determined at the same time. The experiment was performed with 4 different lots each of whole milk and skim milk. The results with a representative lot of each product are shown in table 9.

These results indicate that moderate amounts of developed acidity increased the foaming ability of milk, when the measure-

ments were made at 40°F. Further increases in developed acidity reduced the foaming ability. The decrease apparently is associated with the precipitation of the curd in the milk. When the measurements were made at 80°F., there were no significant variations until considerable amounts of acid had developed; a slight increase was noted then. The measurements made at 140°F. showed no significant variations due to acidity, until enough was present to bring about the formation of a slight curd during the tempering process.

The results with skim milk in general were comparable to those with whole milk.

EFFECT OF CERTAIN ADDED SALTS ON FOAMING ABILITY

Various mixtures were prepared from whole milk, distilled water, and M/2 solutions of each of 5 different salts, as indicated in the table. The mixtures, together with control samples of milk, were stored for a day at about 40°F. The foaming ability of each was determined after storage. The measurements were made in duplicate at 40°, 80°, and 140°F. The results are shown in table 10.

When the measurements were made at 40°F. added calcium lactate reduced the foaming ability of the milk, and added primary magnesium phosphate caused an increase. No significant changes in the foaming ability could be attributed to any other of the salts added, when the measurements were made at 40°F. When the measurements were made at 80°F. no significant changes could be attributed to any of the salts added. When the measurements were made at 140°F. the addition of either calcium lactate or primary magnesium phosphate brought about a decrease in the foaming ability. A partial curdling appeared in these samples when they were tempered to 140°F. The formation of the curd was accompanied by a decrease in the foaming ability.

Sodium citrate, when added in the larger amounts increased slightly the foaming ability of the milk measured at 140°F. Under the conditions of the experiment, added sodium bicarbonate or disodium phosphate showed no effect on the foaming ability of the milk, at any of the temperatures of measurements.

TABLE 10

Effect of various added salts on foaming ability

COMPOSITION OF MIXTURE			PERCENTAGE INCREASE IN VOLUME DUE TO FOAM		
Milk	M/2 SALT SOLUTION	Water	40°F.	80°F.	140°F.
		Calcium lactate			
ml.	*ml.*	*ml.*			
Control	0	0	102	8	27
1900	0	100	101	6	28
1900	40	60	92	6	26*
1900	60	40	80	6	24*
1900	100	0	77	7	23*
		Primary magnesium phosphate			
Control	0	0	98	6	7
1900	0	100	98	6	26
1900	40	60	101	6	24*
1900	60	40	105	6	21*
1900	100	0	117	6	21*
		Sodium bicarbonate			
Control	0	0	95	8	27
1900	0	100	94	8	27
1900	40	60	95	7	27
1900	60	40	95	8	27
1900	100	0	96	7	27
		Sodium citrate			
Control	0	0	102	8	27
1900	0	100	101	6	28
1900	40	60	103	8	29
1900	60	40	104	7	34
1900	100	0	102	8	36
		Disodium phosphate			
Control	0	0	95	8	27
1900	0	100	94	8	27
1900	40	60	95	8	26
1900	60	40	94	8	29
1900	100	0	95	7	28

* Slight curdling during tempering.

SUMMARY AND CONCLUSIONS

A number of the factors influencing the volume of foam appearing on milk were studied. As a measure of the foaming ability of a sample, the "percentage increase in volume due to foam" was determined. The foaming ability of milk varies widely under different conditions. It is necessary, therefore, to specify the conditions under which the measurements are made.

Low temperatures favor the production of the largest volume of foam. With increasing temperature, the volume of foam decreases to a minimum, then rises again with increasing temperature to a second maximum point, lower than the first maximum; this is followed by a decrease in the volume of foam as the temperature rises.

Freshly drawn milk does not exhibit so low a minimum foaming ability as previously cooled and aged milk. The effect of age on the foaming ability of milk seems to be dependent in part on the changes taking place in the fat globules during the aging period. These changes, in turn, are partly dependent on the temperature at which the milk is held.

Agitation of milk at a low temperature reduces its subsequent ability to foam. Agitation at a temperature which is high enough to cause the fat globules to be in a liquid condition increases the subsequent foaming ability of the milk.

There seems to be no definite relationship between the composition and the foaming ability of milk from individual cows. When the influence of the individual characteristics is standardized, increases in the fat content generally decrease the foaming ability of milk, and increases in the milk solids not fat content generally increase the foaming ability.

Moderate amounts of developed acidity increase the foaming ability of milk, as measured at 40°F.; when there is enough developed acidity to cause a curdling of the milk in the cold, the foaming ability decreases. When the measurements are made at 80°F., no significant changes in the foaming ability appear until there is a considerable amount of developed acidity; then a slight increase occurs. When the measurements are made at 140°F., a decrease in the foaming ability is evident as soon as there is

enough developed acidity to bring about a slight curdling of the milk during the tempering process.

When the measurements are made at 40°F., added calcium lactate decreases the foaming ability of milk; added primary magnesium phosphate increases the foaming ability. When the measurements are made at 80°F., none of the added salts as used in this study bring about any change in the foaming ability of milk. When the measurements are made at 140°F., added calcium lactate and primary magnesium phosphate each cause a decrease in the foaming ability; this decrease accompanies a slight curdling of the milk during the tempering process. Added sodium citrate causes a slight decrease in the foaming ability of milk, when the measurements are made at 140°F. Added sodium bicarbonate or disodium phosphate as used in this study have no effect on the foaming ability of milk.

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VARIATIONS IN STREPTOCOCCUS LACTIS*

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The action of *Streptococcus lactis* is of a great deal of importance in the field of dairying because of the frequency with which this species grows in dairy products. Variations in the *S. lactis* organisms are rather common and in milk or cream fermented by them differences in flavor and aroma, consistency, rate of coagulation, etc., are encountered. Many of these differences, although of little importance to the systematic bacteriologist, are very significant in the dairy industry.

It appears that *S. lactis* cultures showing a definite difference in a certain character may sometimes be secured from what would ordinarily be regarded as a pure culture. The work herein reported was done in an attempt to secure, from a pure culture, organisms differing in their response to air supply; it was suggested by the observation that *S. lactis* cultures from a certain source regularly began their development at the tops instead of the bottoms of tubes of litmus milk.

HISTORY OF THE CULTURES STUDIED

One of the extensively used butter cultures contains lactic acid producing organisms that differ from the lactic acid organisms of most butter cultures by beginning the reduction and coagulation of tubes of litmus milk at the tops instead of the bottoms. If the observations are made at the right time, the rather unusual character is very conspicuous since the tubes show reduction and acid production at the tops while at the bottoms there is no evidence of growth. With delayed observations the tubes are reduced, except for a pink band at the top, and coagulated throughout and show nothing unusual. The definite tendency of the organisms to begin their action at the tops of tubes of lit-

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mus milk persists through many transfers and appears to be a well established character.

The cultures employed in the attempts to secure two types of lactic acid organisms from a common source were obtained from the unusual butter culture. They were run through several platings, using whey agar and an incubation of two days at room temperature, with the idea of establishing their purity.

PROCEDURE

The delayed growth of the organisms in the bottoms of the tubes, after rapid development at the surface, suggested that selective inoculations from the bottoms of the tubes before growth had developed too far might yield organisms having somewhat different characters than those secured from the surface.

The cultures from the bottoms were made by means of capillary pipettes having slowly tapering seals so that when the pipettes reached the bottoms the tips could be broken and material drawn into them. After removal from the material being cultured, the pipettes were sealed in a flame and then placed in a 1:1000 aqueous $HgCl_2$ solution to a depth greater than the depth of the milk in which they were used. After several minutes the $HgCl_2$ solution was wiped off with sterile cotton, the tips of the pipettes broken with a sterile forceps and the contents of each blown into a tube of sterile litmus milk. There was, of course, the possibility of carrying material from the surface down into the milk when a pipette was introduced, but the procedure was preferred to the use of special tubes made from test tubes by sealing a straight side arm into each near the bottom and culturing through this side arm. In all cases a culture was made from the surface before the bottom culture was taken so that there was an opportunity to compare the action of the organisms secured from the two portions of a tube.

All of the cultures were grown at a temperature of about 21°C. The freshly inoculated tubes were shaken vigorously to insure distribution of the inoculation material.

RESULTS OBTAINED

Most of the comparisons of material from the top and bottom of a culture were made on tubes that showed a pronounced change in the litmus milk at the surface but no change or only a slight reduction at the bottom. Some of the early attempts gave a conspicuous difference in the cultures from the two sources, those from the tops showing the first growth at the surfaces of the inoculated material and thus resembling the original cultures, while those from the bottoms showed the first growth in the lower portions of the tubes. However, this result was not always obtained and, in a considerable percentage of the trials, both the top and bottom material yielded cultures in which growth began at the surfaces of the inoculated milk. The observations suggested that there was more often a failure to get the two types of cultures when the tubes from which the inoculations were made showed definite reduction at the bottoms than when they did not. In some instances the carrying of surface material down into the milk when the pipettes were introduced may also have been a factor.

When the two types of cultures were secured by selective inoculations, they were frequently carried through series of transfers so that some idea of the stability of the characters could be obtained. The culture showing beginning development at the surface continued to give this change, as would be expected from the persistency of the character in the original cultures. The culture showing beginning development at the bottom usually gave the same change for a number of transfers but then rather gradually varied until it returned to the initial type and began growth at the surface. The time required for the reversion[1] varied considerably with different cultures. With some, only

[1] The change back to the initial type was regarded as a definite reversion, rather than the gradual dominance of the top form in a mixture, for the following reasons:

1. When the top type was known to be present in the material secured from the bottoms of tubes or any other source, it very quickly overgrew the other type and did not require several transfers for its dominance.

2. As pointed out later, the bottom type grew more slowly than the top type and should have been quickly submerged in mixtures of the two.

two or three transfers showed growth at the bottom first, while with others as many as eight transfers were made before the change back to the original type was noted. The effect of the interval between transfers on the time necessary for reversion was studied, using twenty-four- and forty-eight-hour periods. In general, about the same time was required although, of course, this involved twice as many transfers with the short period as with the long.

The cultures which showed the first reduction in the bottom of a tube of litmus milk grew somewhat more slowly than the cultures beginning their action at the top. This was found to be the case whether the inoculation material came from young or old cultures.

From time to time observations were made on the morphology of the two types of organisms. Both commonly showed chains; in young cultures these were often very long, while in older cultures they were shorter but there was still a very definite tendency to chain formation. In general, no difference in morphology between the two types of organisms could be noted, either in cultures showing only beginning reduction or in cultures after coagulation.

DISCUSSION OF RESULTS

From the results secured it appears that the *S. lactis* cultures studied, which were unusual in that they began growth in tubes of litmus milk at the tops instead of the bottoms, could be split into two types, one of which differed definitely from the original cultures by beginning its growth at the bottoms of tubes of litmus milk. Although the variation secured by selective inoculation resulted in a culture that was more like the usual *S. lactis* culture than was the original, it apparently was unstable and, after a number of transfers, reverted to the original type. This suggests that each of the types of organisms encountered can split off from the other. The eventual dominance of the type developing first at the surface of milk is undoubtedly, in part, due to its comparatively rapid growth so that the other type tends to remain submerged to such an extent that it is not com-

monly evident without selective inoculations. Data reported by Hammer[3] show that certain ropy *S. lactis* cultures were split into ropy and non-ropy types by plating and picking colonies. In one instance ropy cultures were secured from a non-ropy one which indicates that this variation was also a reversible one.

The variations that appear to be rather common with *S. lactis* organisms may be a factor in explaining the differences that are encountered among cultures belonging to this species. Probably most of the variations that occur never become conspicuous because of some definite factor, such as competition from the original type, but it would be expected that occasionally the variant would outgrow the other type and thus become of real significance.

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OBSERVATIONS ON ROPINESS IN BUTTER CULTURES*

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Ropiness in milk resulting from the activity of *Streptococcus lactis* is of special importance in the dairy industry because of its occurrence in butter cultures under both practical and carefully controlled laboratory conditions. Cultures which have been normal in consistency for extended periods may develop a ropy condition, either gradually or suddenly, and then on plating commonly yield some *S. lactis* cultures that produce ropiness in milk (*S. lactis var. hollandicus*) (3). The ropy condition in the butter culture may disappear and then reappear later; with certain cultures the appearance and disappearance of the ropiness, at various intervals, continues for long periods without apparent related causes.

From the data available it appears that ropiness in *S. lactis* cultures is not accompanied by a fixed group of other characters (3). This leads to the suggestion that the ropiness may be the result of different types of variations in cultures or, stated conversely, that variations of different types may result in a culture developing ropiness. The data herein reported were secured during the investigation of a ropy butter culture with which the air requirements of the organism seemed to be related to the ropiness.

HISTORY OF THE BUTTER CULTURE STUDIED

The butter culture studied was originally secured by combining *S. lactis* and *Streptococcus citrovorus*. It was carried for several years with very satisfactory results and was sent to many creameries in various parts of the United States. The usefulness of the culture in producing a desirable flavor and aroma in butter is shown by the fact that it was employed in many churnings winning prizes in state and national butter scoring contests.

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After its value was recognized, subcultures were made from time to time and each of these was carried through its own series of transfers with the object of being reasonably certain that the butter culture would not be lost.

In the late summer of 1928, the subcultures that were being carried in the laboratory under very careful conditions suddenly began to show slight ropiness. In some instances transfers sent to creameries developed ropiness, while in others they did not. Later the laboratory transfers showed a pronounced ropiness. Continued transferring finally resulted in the disappearance of the condition and during the nine months that have elapsed since this disappearance there has been no tendency for it to reappear.

Before ropiness developed in the butter culture at the Iowa Agricultural Experiment Station, a transfer was sent to Dr. H. Macy at the Minnesota Agricultural Experiment Station. Doctor Macy reported that, after a considerable number of satisfactory transfers, ropiness appeared in the culture carried by him. This was at about the same time that the ropiness appeared at the Iowa station.

RESULTS OBTAINED

S. lactis organisms present in the ropy butter culture

A transfer of the butter culture which had developed the ropy condition was plated on whey agar and, after incubating the plates at room temperature for two days, 14 *S. lactis* colonies were picked into litmus milk; one of these produced a very ropy condition, while the others showed no ropiness. Another transfer that was plated yielded 2 cultures producing ropiness in milk out of the 33 *S. lactis* cultures picked. Additional attempts to isolate ropy *S. lactis* cultures by plating transfers of the ropy butter culture also indicated that they were much less numerous than the non-ropy cultures and, in some instances, the ropy cultures were entirely missed.

The ropy or non-ropy character of the *S. lactis* cultures isolated appeared to be very definite since, in a number of trials, all the colonies picked into litmus milk from plates poured with one of

TABLE 1

General difference in the development in litmus milk of ropy and non-ropy S. lactis cultures from one source

Inoculated at 8 p.m. Incubation at room temperature

CULTURE	TIME OF INCUBATION						
	13 hours	14 hours	15 hours	16 hours	17 hours	18 hours	19 hours
Ropy 1	Slight reduction at surface	Top $\frac{1}{3}$ reduced	Top $\frac{1}{2}$ reduced	Top $\frac{2}{3}$ reduced	Top $\frac{9}{10}$ reduced	Reduced except pink at surface	
Ropy 2	No change	Slight reduction at surface	Slight reduction at surface	Slight reduction at surface	Slight reduction at surface	Reduced except at bottom	Top $\frac{1}{3}$ reduced, pink at surface
Ropy 3	Slight reduction at surface	Slight reduction at surface	Top $\frac{1}{4}$ reduced	Top $\frac{1}{4}$ reduced	Top $\frac{1}{3}$ reduced	Top $\frac{1}{2}$ reduced	Top $\frac{1}{2}$ reduced
Ropy 4	Slight reduction at surface	Slight reduction at surface	Slight reduction at surface	Reduced at surface	Reduced at surface	Top $\frac{1}{2}$ reduced	Top $\frac{1}{2}$ reduced
Non-ropy 1	No change	No change	No change	Reduced at bottom	Reduced completely	Reduced except pink at surface	
Non-ropy 2	Slight reduction at bottom	Reduced except at surface	Reduced except at surface	Reduced completely	Reduced completely	Reduced except pink at surface	
Non-ropy 3	No change	No change	Bottom $\frac{1}{4}$ reduced	Bottom $\frac{1}{3}$ reduced	Reduced except at surface	Reduced except pink at face	
Non-ropy 4	No change	No change	No change	Slight reduction at bottom	Slight reduction at bottom	Reduced except pink at surface	

the cultures gave the same change in milk as the culture plated. These results indicated that there was no regular transition from either a ropy or non-ropy *S. lactis* type to the other.

Comparisons of the morphology of the ropy and non-ropy *S. lactis* cultures in milk suggested that there was somewhat more of a tendency to chain formation with the ropy than with the non-ropy cultures. The difference was not clear-cut, however, and some chains were present in the non-ropy cultures, while pairs were common in the ropy ones.

There was a conspicuous difference between the ropy and non-ropy cultures in the portion of the tubes of litmus milk in which growth began. While the non-ropy cultures showed the first reduction at or near the bottom of a tube, as is most often the case with *S. lactis*, the ropy cultures began their reduction at the surface. Occasionally with the latter the growth was so definitely limited to the surface for a time that the milk was curdled there when it was not even reduced in the bottom of the tube. The milk inoculated with the ropy cultures eventually curdled throughout just as did that inoculated with the non-ropy cultures.

The general difference in the development of the two types of cultures in tubes of litmus milk is shown in table 1. The cultures were inoculated at 8 p.m., the inoculated milk thoroughly shaken and observations made the next day; incubation was at room temperature. From the results given, it is evident that the two types of cultures differed very definitely in the portions of the tubes in which growth occurred first. The non-ropy cultures began the reduction at the bottom and extended it from that area while the ropy cultures began their growth at the surface and then developed downward. Additional observations, also made under comparable conditions, confirmed the general variation shown in table 1.

Selective transfers of material from the butter cultures

The tendency of the ropy *S. lactis* cultures to develop at the surface suggested attempts to isolate them from the butter culture by a series of transfers, using surface material for the inoculations. Because the butter culture had lost its ropy

character, it was necessary to try this procedure on a non-ropy transfer. It was inoculated into a tube of litmus milk and, after coagulation, a series of transfers through tubes of litmus milk was begun, the inoculation material being taken regularly with a needle from the very surface of the fermented milk. The transfers were made every day or two, depending on the rate of coagulation, and were incubated at room temperature. After somewhat more than a month of such transferring, slight ropiness was noted, soon there was evidence of reduction beginning at the surface and then pronounced ropiness developed. On continued transferring the ropiness and early surface growth were maintained through many inoculations.

In another more extended trial, that was also begun with the butter culture showing no evidence of ropiness, room temperature and 21°C. were used and at each temperature one series of transfers involved inoculations with a needle from the surface of the fermented milk, while in another series the transfers were made by dipping a loop well down into the milk. At room temperature the eighth surface transfer was definitely ropy and at about the same time reduction regularly began at the top. This combination of characters persisted through the remainder of the 88 transfers made, although some of the later inoculations involved the transferring of material several days old. None of the 88 deep transfers made at room temperature became ropy or began the reduction of the milk at the surface. Microscopic examinations showed that after a number of transfers there was much more of a tendency to chain formation with the organisms in the material secured by surface inoculations than in that secured with deep transfers. Volatile acid determinations were made on material from the two series after 75 inoculations of each; the method employed was the one regularly used at the Iowa Agricultural Experiment Station (2). The value for the surface material was 2.9[1] and that for the deep material 20.7; these

[1] This value represents the cubic centimeters of N/10 NaOH required for the neutralization of the first 1000 cc. of distillate obtained when a 250-gram portion of the fermented milk was distilled with steam after the addition of 15 cc. of approximately N/1 H_2SO_4.

results indicate that one of the volatile acid producing types associated with *S. lactis* in butter cultures was still present in the deep material but not in the surface material.

With the transfers made at 21°C. the surface material showed ropiness on the ninth transfer and reduction at the top before the bottom began at about the same time. This change in character continued through the 85 transfers made. None of the 85 deep transfers developed ropiness and reduction of the litmus continued to begin at the bottom. There seemed to be little if any difference in the morphology of the organisms in the two series of transfers. Volatile acid determinations made after 79 inoculations in each series gave a value of 2.9 for the surface material and 23.0 for the deep material.

Although the butter culture continued to be normal in consistency, after several months further transfers were made at 21°C., one series involving the inoculation of material with a needle from the surface, while the other involved the inoculation of material with a needle from throughout the mass of fermented milk. After 45 transfers both procedures gave only a non-ropy fermentation, with reduction regularly beginning at or near the bottom. There seemed to be a tendency for the chains in the surface material to be somewhat longer than those in the other material. When these two series had been through about 35 inoculations, a report that a transfer of the butter culture had become ropy in a creamery suggested another trial, using the same procedure. After 10 transfers, which was a greater number than was at one time required to secure a ropy culture, there was no evidence of ropiness or of reduction beginning at the surface. Microscopic examinations indicated that the morphology of the organisms in the two series was quite the same.

The effect of transferring from the surface only on a number of other butter cultures was also studied. The first culture used was one which had been carried in the laboratory for several years because of a tendency to develop and then lose the ropy character. It was sent to the laboratory by a creamery organization desiring information on the cause of the ropiness. Transfers were begun when it was not ropy, the inoculation material

being taken with a needle from the surface only. After a number of transfers, the culture changed so that reduction began at the surface but ropiness was never noted, although 88 transfers were made. Three other butter cultures of a good quality, none of which had ever been ropy, were each carried through two series of transfers at 21°C.; one series involved the inoculation of surface material, while with the other the transfers were made by plunging the needle deep into the fermented milk. Although each series was continued through 32 transfers, ropiness and beginning surface reduction failed to appear with any of the three. With one culture the inoculation of surface material tended to increase the chain formation, while with the other two no variation in morphology accompanied the change in the method of inoculation.

Attempts to secure non-ropy from ropy cultures

The slow progression of growth from the surface downward, with some of the ropy *S. lactis* cultures isolated, suggested the possibility of securing non-ropy cultures from the ropy ones by taking freshly reduced material from the bottom of a culture. Special tube for this were prepared from test tubes by sealing a straight side arm of about 5 mm. inside diameter into each near the bottom. Litmus milk was sterilized in these tubes and inoculated through the main tube. When growth had progressed to the desired point, material was taken from the bottom of the tube by means of a capillary pipette inserted through the side arm; control inoculations were also made from the surface of the main tube.

In a number of instances material taken from the bottom of a tube gave a growth in litmus milk without ropiness, while the controls were definitely ropy. Other attempts, however, gave a ropy culture from both the top and bottom materials. In general, it appeared that if the reduction in the bottom of a tube was complete when the inoculation from it was made, the culture secured was more likely to be ropy than if reduction was not so advanced. The ability of the ropy type of *S. lactis* to grow at the bottom of a tube was also evident from the pronounced ropiness

developing in the side arm when the incubation period was prolonged.

Some of the non-ropy cultures secured were carried through a number of transfers without ropiness appearing, while others soon developed a ropy condition. When ropiness appeared, it was usually slight in one transfer and then more pronounced in a later one.

DISCUSSION OF RESULTS

The isolation of ropy *S. lactis* cultures (*S. lactis var. hollandicus*) from a ropy butter culture is in agreement with the evidence from various sources indicating that ropiness in butter cultures is most often due to a ropy *S. lactis* organism instead of to one of the types commonly related to ropiness in sweet milk. The ropy organism isolated, however, differed from most *S. lactis* strains in the tendency to begin the reduction of tubes of litmus milk at the surface instead of at the bottom.

The development of ropy cultures by selective inoculations from the surface of a butter culture that had lost its ropiness, after a period when this character was regularly evident, was undoubtedly due to the correlation between the ropy condition and the air requirements. The ropy cultures secured were apparently cultures of *S. lactis* only, since volatile acid determinations showed that the volatile acid producers associated with *S. lactis* in butter cultures were not present. Presumably the ropy *S. lactis* organism, after a period when it was sufficiently numerous to cause ropiness in the butter culture, decreased to a point where the ropiness disappeared. For a time after this decrease, the organism could still be isolated by selective inoculation, which was easily carried out because of the unusual air relationship, but later it had either entirely disappeared or decreased to such a point that the selective procedure failed.

The development of non-ropy cultures from ropy ones by using material showing beginning reduction in the bottoms of the tubes of litmus milk again shows the correlation between the ropiness and the air requirements. It further illustrates the splitting off of a non-ropy culture from one that is definitely ropy and, in this

respect, confirms the work of Hammer (1) who found it possible to secure both non-ropy and ropy *S. lactis* organisms by picking colonies from plates poured with certain ropy *S. lactis* cultures.

Two explanations for the original presence of *S. lactis var. hollandicus* in the butter culture suggest themselves; first, contamination and, second, a change in the character of an organism already there. Contamination does not seem probable because of the other butter cultures that remained normal when handled under the same conditions and also because the most logical sources of contamination with butter cultures would not be expected to supply *S. lactis* of any type. In the work reported by Hammer (1), ropy *S. lactis* cultures were secured in one instance by plating a non-ropy culture and picking colonies into litmus milk, and such a sudden change in the character of an organism seems the most logical explanation in the case of the butter culture studied. There would not need to be a change in a large number of cells because a variation in a few or even one cell could become of importance through reproduction.

Presumably a change in the character of an organism has a definite cause. In the instance studied, the factor responsible for the ropiness seems to have definitely influenced the air requirements and since in at least some instances the ropy cultures showed more chain formation than the non-ropy ones, it may have affected the morphology.

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MINUTES OF MEETING OF ADVANCED REGISTRY SECTION, AMERICAN DAIRY SCIENCE ASSOCIATION

HOTEL MISSOURI, ST. LOUIS, MISSOURI

8:15 P.M. October 14, 1929

Chairman, J. R. DICE *Acting Secretary*, K. S. MORROW

Minutes of last meeting read and approved. The chairman appointed the following nominating committee: W. E. Peterson, Minnesota; R. T. Harris, Wisconsin.

Professor J. B. Fitch, chairman of the Breeds' Relations Committee, gave the following report:

REPORT OF THE BREEDS' RELATIONS COMMITTEE MEETING HELD OCTOBER 13, 1929; ST. LOUIS, MISSOURI

The Summary of answers to letters in regard to Advanced Registry rules was read and discussed. The comment of breed association officials to this letter was not enclosed in the letter sent to the supervisors of Advanced Registry. Some of the comments were as follows:

1. That the no talking rule was not being enforced in many of the states.

2. That there was a wide difference in the method of handling finances at the different schools and a lack of business methods in handling accounts at some institutions. It was brought out that the Jersey and Guernsey breeds guarantee payments up to three months, while the Ayrshire and Holstein breeds extend this to six months.

3. That the preliminary milking was not recorded or tested in many states.

If a state has adopted the uniform rules of the American Dairy Science Association there should be no question about the enforcement of rules covering comments 1 and 3 above.

A committee is to be appointed to make recommendations

regarding the guarantee for payment of testing. This matter is becoming more complicated due to the adoption of the bi-monthly test.

The Breeds' Relations Committee approved the use of the bimonthly test and suggested that the breed associations consult with the representatives of the American Dairy Science Association in formulating their rules for this test.

R. T. Harris of Wisconsin reported for the committee on the uniform blank for herd tests. A uniform blank has been agreed upon and will be printed, along with the two-day blanks. As the supply of two-day report blanks is low, plans were made to have a new supply of this blank and the uniform herd test blank printed in the near future.

Motion made by J. E. Wylie, Tennessee, and seconded by R. B. Becker, Florida, that report of Breeds' Relations Committee be approved and adopted as reported. Motion carried.

Warren Gifford, Missouri, presented a paper entitled, "A Statistical Study of the Reliability of Bimonthly Tests."

Lynn Copeland, American Jersey Cattle Club, presented a paper entitled, "The Bimonthly Test," Mr. Copeland reported that 77 Jersey breeders were at the present time using this test.

The meeting was opened for discussion of the papers presented by Gifford and Copeland. Eight states reported the use of the Bimonthly test.

H. W. Norton, Jr., Holstein-Friesian Association of America, explained briefly the purpose and operation of the Holstein Plan of Herd Classification. He reported that 63 herds, totaling 1800 animals, had been classified to date.

Wm. Moscrip, Holstein-Friesian Association of America, led the discussion following Norton's remarks. He especially mentioned the aim of the Holstein-Friesian Association to eliminate from the Holstein breed those animals falling in the two lower classifications of the plan.

The Nominating Committee reported the following names:

Chairman: A. A. Borland, Pennsylvania; E. L. Anthony, Michigan.

Secretary: P. C. McGilliard, Oklahoma; A. F. Kuhlman, Illinois.

J. E. Wylie, Tennessee, moved and C. L. Allen, New York, seconded the adoption of the report of Nomination Committee. Motion carried.

The following were elected: Chairman, E. L. Anthony; Secretary, P. C. McGilliard.

THE RELIABILITY OF BI-MONTHLY TESTS*

WARREN GIFFORD†
University of Missouri

The problem of obtaining an accurate quantitative measure of the dairy cow's inherited producing ability has occupied the attention of dairymen since very early times. According to Morse (1), perhaps Pyrrhus, in 300 B.C., who measured milk from cows that produced approximately 40 liters per day was one of the first to realize the importance of such a measure. The great developments in tests and measures that have adapted themselves to such a purpose have been made during the past half century.

The strictly official plan of testing, as accepted at the present time by the Holstein-Friesian Association of America (2), has met the requirements as a test that is accurate for the period of time that it is put into operation and has eliminated to a great extent, fraudulent practices that may occur in the making of records.

Although the strictly official test plan has met the requirements of an accurate quantitative measure of the dairy cow's producing ability under certain conditions of management and feeding, it has not met with favor among dairy cattle breeders. It has been shown that short time official records are of relatively little value in predicting a cow's milk and butterfat production for a complete lactation period, and the enormous costs incurred in the supervision of the long-time strictly official test have made it an impractical measure of milk and butterfat production.

In order to reduce the cost of testing, breed associations have adopted plans of supervision that determine the amounts of butterfat produced during a short interval each month, i.e.,

* Received for publication August 23, 1929.

† The author is greatly indebted to H. W. Norton, Jr., Superintendent of Advanced Registry, Holstein-Friesian Association of America, for his assistance in securing a large number of the records studied; and to Professors C. W. Turner and A. C. Ragsdale, University of Missouri, for their many helpful suggestions.

one and two days, and at which time considerable effort is expended in determining whether or not the records are honestly made and accurately reported, and from these data the amounts of fat produced during the lactation period have been estimated. The reliability of these tests has been studied by Yapp (3), Petersen (4), and others, and it was shown that the errors of estimate were rather small but that considerable variations frequently occurred.

These forms of records, known as semi-official records, have been great factors in the improvement of pure bred dairy cattle. Since there are twelve or more supervisions during the lactation period, there has been considerable expense to the breeders who have conducted these tests, and no doubt many breeders of purebred dairy cattle have not been able to meet the financial obligations entailed in testing. Therefore, some of the advancement that could have been made in the improvement of dairy cattle has been curtailed.

It has been suggested from time to time that any method of supervision of yearly tests that would reduce the cost of testing, give an accurate estimate of the yearly production, and still not lower the standards of the present plans of testing by giving greater opportunity for fraudulent practices, would act as a stimulus to the amount of testing.

McDowell (5) and Copeland (6) have presented data to show the relative reliability of yearly records that have been estimated from six supervisions per year. Gaines (7) has shown that when the short-time official test is conducted during the fifth month of the lactation period, it is of greater reliability than other short time official tests. Saiz (8) has also presented a plan of three tests per year that will estimate the annual production with considerable accuracy.

Copeland selected at random 250 Register of Merit records made under monthly two-day supervision and 250 records that were made by the one-day supervision; and computed the yearly butterfat records by using only the alternate regular tests and the automatic retests which those tests occasionally required. In 50 per cent of the cases the first test made after the starting

of the lactation period was used as a beginning and in the other 50 per cent the second test was used. He found that the average variations were as follows:

250 records (two-day supervision)	6.73 pounds fat 0.901 per cent
250 records (one-day supervision)	7.69 pounds fat 1.53 per cent
500 records (one- and two-day supervision	7.21 pounds fat 1.21 per cent

From the study of these data he concluded that records that were made every other month under supervision show little variation from those made by the usual monthly supervision. McDowell drew similar conclusions from his study of Cow Testing Association records.

In view of the findings of the studies of the Register of Merit records, the American Jersey Cattle Club adopted the plan of Bi-monthly testing. Other breed associations have been considering the adoption of a similar plan of Advanced Registry.

Therefore, these interests have inspired the writer to make some investigations as to the reliability of butterfat records obtained by the bi-monthly plan of testing as compared to the present form of monthly testing for yearly records.

The records studied were obtained by selecting at random 100 or more Advanced Registry records for Holstein and Guernsey cows tested monthly by the two-day test plan for each of the following groups of yearly butterfat production:

300 to 399.9	700 to 799.9
400 to 499.9	800 to 899.9
500 to 599.9	800 to 999.9
600 to 699.9	1000 and over

It was believed that the selection of records representing definite classes of production was of special value in getting data that would be representative of all breeds and such that would show the reliability of the method of testing for records of cows at various productions.

From these data yearly records were estimated by applying the bi-monthly test plan, using only alternate tests. The records obtained by using the tests for the first, third, fifth, seventh, ninth, and eleventh months were called the "odd month records" and those that were calculated by starting the test with the second month of the lactation period and continuing by using the

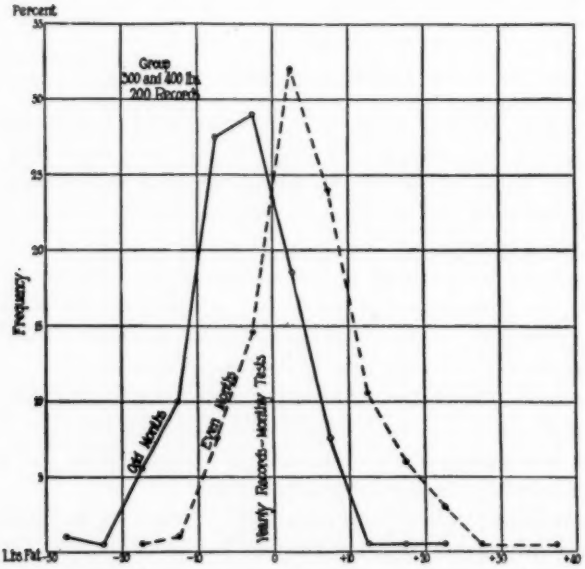

FIG. 1. DISTRIBUTION OF THE DEVIATIONS IN POUNDS OF FAT BETWEEN MONTHLY AND ESTIMATED BI-MONTHLY RECORDS IN THE 300- AND 400-POUND GROUP

fourth, sixth, eighth, tenth, and twelfth month tests, respectively, were called the "even month records."

For convenience, these records were combined into four groups as follows: those between 300 and 499 pounds, 500 and 699 pounds, 700 and 899 pounds and those above 900 pounds.

In order to compare the reliability of the estimated bi-monthly yearly records as calculated, the deviations in pounds of fat from the records as determined by the monthly tests were determined,

and the frequencies plotted, and are shown in figures 1 to 5, inclusive.

The standard errors of estimate were determined and the correlation coefficients between the actual records and the estimated records were computed for all the groups and for the group representing all records studied, and are given in table 1.

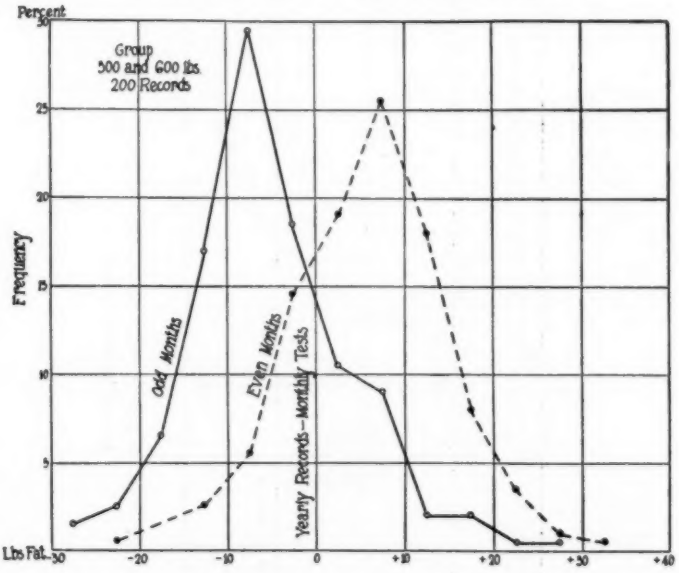

FIG. 2. DISTRIBUTION OF THE DEVIATIONS IN POUNDS OF FAT BETWEEN MONTHLY AND ESTIMATED BI-MONTHLY RECORDS IN THE 500- AND 600-POUND GROUP

The value of the means for these groups indicate whether or not the estimations are satisfactory in determining the yearly butterfat records. The standard error of estimate is a measure of the prediction value of the method used for estimating the production and indicates the variation that may be expected in the application of the method. About 69 per cent of all of the bi-monthly records will lie within the range of the standard error of estimate, 95 per cent will fall within two times the standard

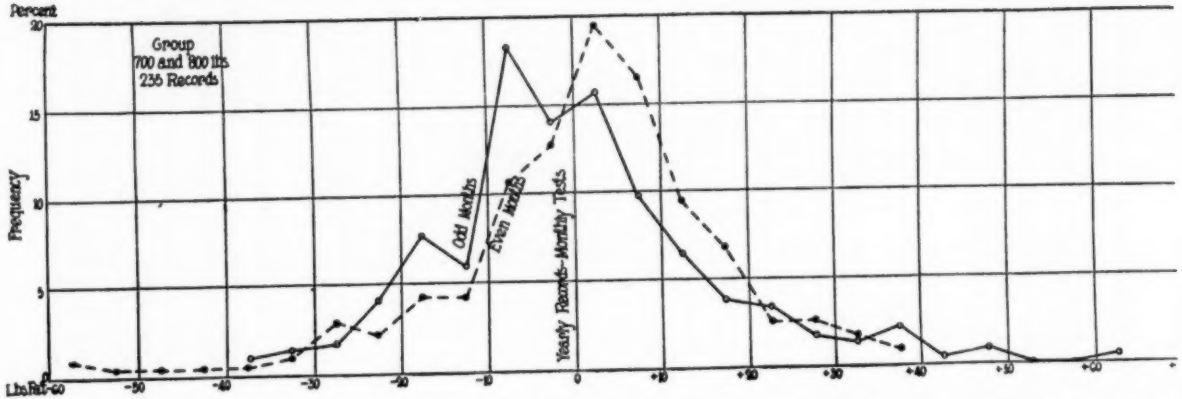

FIG. 3. DISTRIBUTION OF THE DEVIATIONS IN POUNDS OF FAT BETWEEN MONTHLY AND ESTIMATED BI-MONTHLY RECORDS IN THE 700- AND 800-POUND GROUP

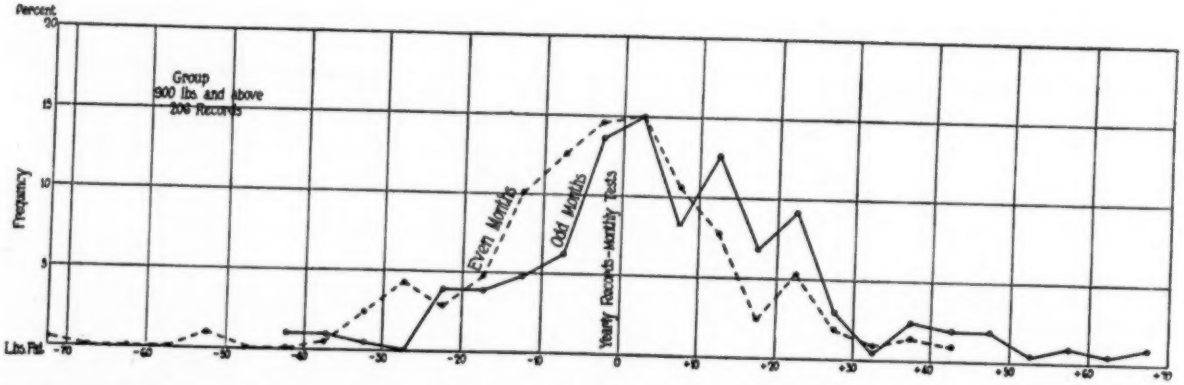

FIG. 4. DISTRIBUTION OF THE DEVIATIONS IN POUNDS OF FAT BETWEEN MONTHLY AND ESTIMATED BI-MONTHLY RECORDS IN THE 900 POUNDS AND ABOVE GROUP

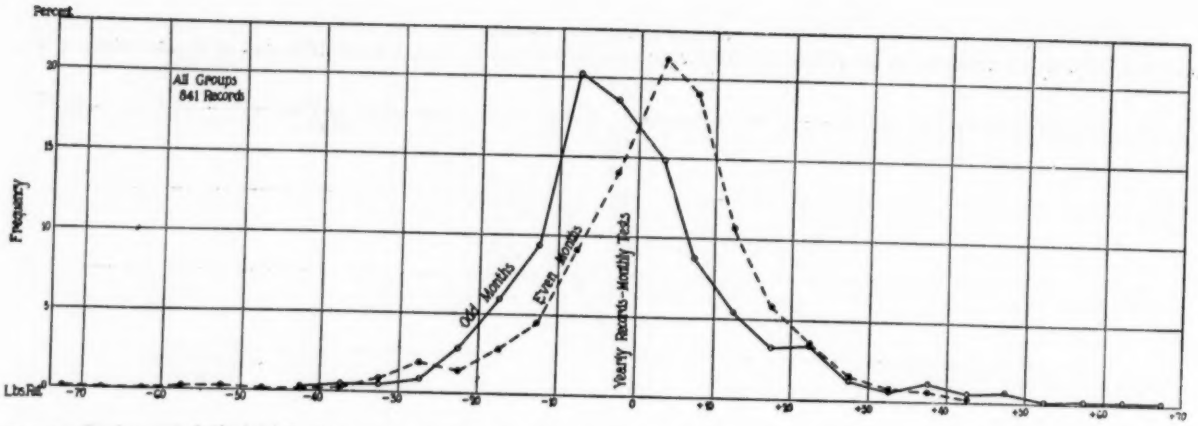

FIG. 5. DISTRIBUTION OF THE DEVIATIONS IN POUNDS OF FAT BETWEEN MONTHLY AND ESTIMATED BI-MONTHLY RECORDS IN ALL GROUPS OF RECORDS

error of estimate, and 99.7 per cent will fall within three times the standard error of estimate. Therefore, the smaller the standard error of estimate the less the dispersion about the line of the actual records.

An examination of the data presented in table 1 shows that the means of the deviations from the actual records for the odd month for the 300 and 400 pound groups are -4.12 ± 0.371 and

TABLE 1

Means, variables, and coefficient of correlation between yearly butterfat records estimated by the bi-monthly test plan from the records obtained by monthly tests

FACTORS	NUMBER YEARLY RECORDS	CLASS		MEAN OF THE DEVIATION	STANDARD ERROR	COEFFICIENT OF CORRELATION r
		pounds		*per cent*		
Monthly bi-monthly records	200	300, 400	Odd	-4.12 ± 0.347	7.27 ± 0.245	*0.981 ± 0.002
			Even	$+4.82 \pm 0.371$	7.78 ± 0.262	0.991 ± 0.001
	200	500, 600	Odd	-5.12 ± 0.435	9.12 ± 0.308	0.982 ± 0.002
			Even	$+5.80 \pm 0.427$	8.95 ± 0.302	0.986 ± 0.007
	235	700, 800	Odd	-0.1 ± 0.694	15.78 ± 0.491	0.972 ± 0.003
			Even	$+0.97 \pm 0.667$	15.16 ± 0.472	0.973 ± 0.002
	206	900 and above	Odd	$+7.33 \pm 0.894$	19.02 ± 0.632	0.956 ± 0.004
			Even	-1.28 ± 0.767	16.32 ± 0.542	0.971 ± 0.003
	841	All groups	Odd	-4.90 ± 0.346	14.88 ± 0.244	†0.986 ± 0.001
			Even	$+2.50 \pm 0.375$	13.23 ± 0.218	0.997 ± 0.001

Class interval:
* 10.0 pounds.
† 20.0 pounds.

-5.80 ± 0.427 pounds fat, and $+4.82 \pm 0.371$ and $+5.80 \pm 0.427$ pounds fat for the even month groups, respectively, and a total difference of 8.9 and 10.9 pounds of fat between the means of estimated records calculated on odd and even months. The standard errors of estimate are relatively small, being 7.275 ± 0.245 and 9.123 ± 0.308 pounds for the odd month records and 7.782 ± 0.262 and 8.950 ± 0.302 pounds for the even month records.

The means for the 500 and 600 pound groups indicate that the method used in predicting the yearly records was even more satisfactory since the means of the deviations for the odd month records are -0.1 ± 0.694 and 7.33 ± 0.894 pounds and for the even months $+0.97 \pm 0.667$ and -1.28 ± 0.767 pounds, respectively. The standard errors for these groups were 15.980 ± 0.491 and 19.015 ± 0.632 pounds for the odd month records and 15.165 ± 0.472 and 16.315 ± 0.542 pounds for the even month records.

When all of the data are combined, there is a mean of -4.9 ± 0.346 pounds fat for the deviations of the records estimated by the odd month test and $+2.499 \pm 0.375$ pounds fat for those estimated by using the even month tests.

The coefficients of correlation indicate that there is a significant degree of relationship between all groups of estimated records and the yearly records obtained by the monthly plan of testing.

Therefore, it seems, according to the above data, that the bi-monthly method of estimating the yearly fat production of dairy cows is satisfactory, when such records are made with the same degree of accuracy and honesty as are those records made by the monthly test plan.

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THE EFFECT OF LACTOSE ON THE SURVIVAL OF ESCHERICHIA COLI WHEN HEATED TO 145°F. FOR THIRTY MINUTES*

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In commercial pasteurization the evidence is conclusive that members of the Escherichia-Aerobacter group may survive in milk that has been heated to approximately 145°F. for a period of thirty minutes. This temperature has been officially accepted as being sufficiently high to insure the safety of pasteurized milk.

It is rather uncertain whether the survival of members of this group is due to defective pasteurizing equipment, to the physiology of the organism, or to certain biochemical influences of the milk itself. Considering the colloidal state and chemical constituents of milk, it would seem possible that a protective action may at times be established.

Of the chemical constituents in milk lactose offers the best possibility for investigation. This sugar is present in cow's milk in amounts varying between 4 and 5 per cent.

The available literature on this subject supports the evidence herewith presented. Robertson[1] states that,

> Hypertonic solutions, as indicated by comparisons of heating cells in nutrient broth containing increasing concentrations of sucrose, have a protective action up to and including 50 per cent sucrose. The protective action may be explained, in part, by the osmotic influence of the sucrose solution outside of the cell wall, and, in part, by the presence of capsules formed about the cells when growh in the sucrose solutions.

EXPERIMENTAL

Forty-eight hour broth cultures of the strains to be tested were made from agar slants. One-tenth cubic centimeter quantities

* Received for publication August, 1929.

[1] Robertson, A. H. 1927 Thermophilic and thermoduric microorganisms with special reference to species isolated from milk. N. Y. State Agr. Exp. Sta. Buls. 130–131; Vt. Agr. Exp. Sta. Buls. 274–275.

were inoculated into tubes of broth containing 0, 1, 2, 3, 4, 5, 7.5, 9.5, 12.5, 15, and 20 per cent lactose and then incubated at 37°C. for forty-eight hours. One-tenth cubic centimeter quantities were then inoculated into corresponding tubes of lactose broth and pasteurized at 145°F. for thirty minutes in a water bath. The tubes were removed, cooled, and the contents of each poured into fermentation tubes containing 2 per cent lactose

TABLE 1

Influence of lactose on the survival of Escherichia coli heated to 145°F. for thirty minutes

+ growth; − no growth

	TEST NUMBER	0	1 PER CENT	2 PER CENT	3 PER CENT	4 PER CENT	5 PER CENT	7.5 PER CENT	9.5 PER CENT	12.5 PER CENT	15 PER CENT	20 PER CENT
Strain 1	1	−	−	−	−	+	+	+	+	+	+	+
	2	−	−	−	−	+	+	+	+	+	+	+
	3	−	−	−	−	+	+	+	+	+	+	+
	4	−	−	−	+	+	+	+	+	+	+	+
	5	−	−	+	+	+	+	+	+	+	+	+
	6	−	−	−	+	+	+	+	+	+	+	+
	7	−	−	−	−	+	+	+	+	+	+	+
	8	−	−	−	+	+	+	+	+	+	+	+
	9	−	−	−	−	+	+	+	+	+	+	+
Strain 2	1	−	−	+	+	+	+	+	+	+	+	+
	2	−	−	−	+	+	+	+	+	+	+	+
	3	−	−	−	−	+	+	+	+	+	+	+
Strain 3	1	−	−	−	−	+	+	+	+	+	+	+
	2	−	−	+	+	+	+	+	+	+	+	+
	3	−	−	−	−	+	+	+	+	+	+	+

broth. In addition, 1 cc. portions from each tube were plated on lactose agar. Results were recorded after ninety-six hours incubation at 37°C.

DISCUSSION

Table 1 presents data showing the protective action of increasing concentrations of lactose on the survival of *Escherichia coli*

when heated to 145°F. for thirty minutes. A protective action is established when these organisms are grown in broth containing from 4 to 20 per cent lactose. In several cases survival occurred in as low as 2 or 3 per cent solutions of lactose. As the percentage of lactose in milk varies between 4 and 5 per cent a similar phenomenon may occur in pasteurized milk.

The thermal death-points of the strains used had been determined to be between 142° and 143°F. for thirty minutes. The tubes of broth containing no lactose acted as controls. Comparing these temperatures with the one at which survival occurred, that is, 145°F. for thirty minutes, it is seen that the increasing concentrations of lactose may cause an increase in the death-point temperatures of at least 2° or 3°. This factor may prove to be vital in commercial pasteurization.

The data in table 1 are based on 15 determinations. Three trials not included showed this protective action but with breaks.

The findings indicate that the greater the concentration up to and including 20 per cent lactose, the greater the probability for survival. This fact may be of value in explaining the survival of members of the Escherichia-Aerobacter group in pasteurized ice cream mixes which usually contain from 10 to 15 per cent sugar. It should be noted that the sugar used in ice cream mixes is sucrose but the same protective action may occur due to the fact that lactose and sucrose are isomeric.

CONCLUSION

1. The ability of *Escherichia coli* to survive pasteurization when grown in increasing concentrations of lactose suggests the possibility of a similar protective action in milk.

2. This survival of *Escherichia coli* seems to bear a relation to the concentration of lactose up to and including 20 per cent.

3. Survival of members of the Escherichia-Aerobacter group in pasteurized ice cream mixes may be caused by the protective action of the high sugar content.

THE ESCHERICHIA-AEROBACTER GROUP AS AN INDEX TO PROPER PASTEURIZATION*

E. ARTHUR BEAVENS

Department of Bacteriology, University of Maryland, College Park, Maryland

Several investigators have attempted to use the survival of certain organisms in milk as an index to the efficiency of pasteurization. The coli bacillus, because its thermal death-point more closely approaches the pasteurization temperature, has been often used in this capacity. Other investigators have been reluctant in accepting the data offered to support the coli index of pasteurized milk. The problem is still in a state of controversy because of the need of additional work.

Several influencing factors are brought into prominence when heat, especially high temperatures, is applied to protoplasmic matter. Cell metabolism is materially increased resulting in the possible formation of a resistive cell wall, an alteration of the protoplasm, or a rearrangement of the molecular structure of the cell. Acclimatization of bacteria to high temperatures may occur if successive generations are grown from those having high absolute thermal death-points or those possessing true heat-resistance such as the thermophiles. It is logical that the effects of these factors must be thoroughly investigated and considered before any relationship can be established between the coli count and the efficiency of pasteurization.

REVIEW OF LITERATURE

Coli bacilli with high thermal resistances have been observed by De Jong and De Graff (7) who described seven strains of *E. coli* which survived 149° to 152.6°F. for thirty minutes in milk or broth. One strain withstood 185°F., frequently it survived 140° to 149°F. for thirty minutes. The culture was more easily killed in bouillon than in milk. They concluded from these

* Received for publication August, 1929.

results that the presence of *E. coli* in pasteurized milk could not be taken as an index of proper pasteurization. Gage and Stoughton (3) showed that the majority of thermal death-points of *E. coli* were from 132° to 140°F. The temperature at absolute death-point showed the presence of heat resistance organisms in this group. Zelenski (12) also found a strain of *E. coli* which survived unusually high temperatures.

Ayres and Johnson (2) observed that the thermal death-points of 174 cultures of the coli bacillus isolated from cow feces, milk, cream, human feces, flies, and cheese, varied considerably when heated in milk for thirty minutes. At 140°F., 95 cultures (54.69 per cent) survived. One culture was not killed at 150°F. on one occasion only. All cultures had low majority death-points, survival was due to the resistance of a few cells. This fact complicated the coli test as an index to the efficiency of pasteurization. They state that the coli test may be of value if the milk is pasteurized at 150°F. for thirty minutes.

Ayres and Johnson (1) pointed out the relationship between the "majority" and "absolute" death-points of bacteria in relation to pasteurization. These investigators found the "majority" death-point of *A. aerogenes* to be 135°F. for thirty minutes, while the "absolute" point was a little above 150°F. for thirty minutes. When 135°F. was used surviving cells could be detected by examining 1 per cent of the volume pasteurized, when 140°F. was used 2 per cent of the volume was tested in order to find those surviving: at 145°F. no living organism could be detected in 1 cc. or even when 14 per cent was tested, surviving cells were found in the remaining 85 per cent.

Tanner (11) states that,

> Although neither the *Escherichia coli* (*Bacterium coli*) cultures nor the *Serratia marcescens* (*Bacillus prodigiosus*) strains used proved to be very good as indicators of efficiency of pasteurization by the holder process, for several reasons the *Escherichia coli* (*Bacterium coli*) would probably prove to be more accurate as such an indicator. It seems fair to assume, in view of the results obtained, that *Escherichia coli*, in concentrations in which it occurs in milk, would be destroyed by exposure to 62.8°C. for thirty minutes, yet there is always the possibility

of encountering resistant strains or cultures containing some resistant cells.

SCOPE OF PROBLEM

The scope of this paper includes the determination of the presence or absence of organisms of the Escherichia-Aerobacter group in raw milk and in milk pasteurized under commercial conditions. Data were collected to determine whether or not coli bacilli survived the pasteurization temperatures.

Part of the studies were performed with pure cultures under laboratory conditions so actual associative and environmental conditions were disregarded. The conclusions may be, as stated by Marshall, "Like studying man apart from society in order to obtain his social relations." However, until better methods of technique are perfected to obtain results under actual conditions, we must rely on laboratory tests and pure cultures to assist us in obtaining the information that we are seeking.

All samples of milk were obtained from the dairy of the University of Maryland. The experiments were conducted from August, 1927, to April, 1929 under the supervision of Dr. E. M. Pickens, Head of the Department of Bacteriology.

METHODS AND PROCEDURE

The samples of milk to show the efficiency of pasteurization were taken from the vat before and immediately after the heating process. Pasteurization was accomplished by heating in a Pfaudler glass-lined pasteurizer to approximately 145°F., holding for thirty minutes and then cooling to about 50°F.

These samples of milk were plated on standard agar in the following manner: the raw milk in dilutions of 1:100; 1:1000; 1:10,000; the freshly pasteurized milk in dilutions of 1:10; 1:100; and 1:1000. An Endo's plate was made from each sample in a dilution of 1:10. Ten cubic centimeters, 1 cc.; and 0.1 cc. portions of milk from each sample were inoculated into fermentation tubes containing 2 per cent lactose. All plates and tubes were incubated at 37°C. for forty-eight hours.

The agar used was the standard beef extract media adjusted

to a pH of 6.5 to 6.6 as recommended by Standard Methods of Milk Analysis (10). The Endo's media was prepared from the formula recommended in Giltner's Microbiology (4).

The technique used to determine the possible acclimatization of coli organisms to high temperatures consisted of two parts; the determination of the thermal death-points of the strains to be used, the actual acclimatizing process. In the first part, characteristic coli colonies were isolated from raw milk plates and replated several times to insure the purity of the cultures. These strains were then inoculated into dextrose, sucrose, lactose, and mannite broths to test their gas-forming abilities.

To differentiate these strains as fecal or non-fecal types, the Voges-Proskauer and methyl red reactions were used. The first test was performed by growing the strains in 5 cc. quantities of Bacto M. R.-V. P. medium for twenty-four hours after which equal amounts of 10 per cent KOH were added. Fecal types are Voges-Proskauer negative. The methyl red tests were performed by growing the strains in 5 cc. quantities of Bacto M. R.-V. P. medium for forty-eight hours after which 5 drops of methyl red were added.

To determine the thermal death-points of the selected strains the open tube method was used. A forty-eight hour broth culture was made and 0.1 cc. quantities inoculated into tubes of litmus milk. Five tubes of the inoculated litmus milk were placed in a water bath adjusted well below 145°F. and held at this temperature for thirty minutes. At the end of the heating period the tubes were removed and cooled in tap water. The water bath was then adjusted 2° higher and five tubes heated for a like period. This procedure was repeated until a temperature well above that used in pasteurization had been reached. All tubes were incubated at 37°C. for approximately a week. Those tubes showing a red color due to acid production were recorded as positive, those having no color change as negative.

The acclimatizing process, or the second part, was accomplished by preparing a forty-eight hour broth culture and then inoculating 1 cc. quantities into each of 5 tubes of broth. These tubes were then immediately heated in a water bath at 142°F. for thirty

minutes, after which they were removed and cooled in tap water. One cubic-centimeter quantities were then taken from each tube and inoculated into five tubes each of broth and litmus milk. These tubes were then incubated at 37°.C for forty-eight hours. If growth occurred in the broth and the litmus milk tubes, 1 cc. quantities were taken from each of the broth tubes and reinoculated into 5 tubes of the same medium. These were then heated to 143°F. for thirty minutes and the same procedure repeated. This was continued until the culture was finally killed which

TABLE 1

A percentage comparison between the reduction of the total count and coli count of raw and pasteurized milk

TOTAL COUNTS			COLI COUNTS		
Percentage reduction		Number of samples	Percentage reduction		Number of samples
100–95	=	51	100–95	=	84
94–90	=	22	94–90	=	9
89–85	=	12	89–85	=	2
84–80	=	5	84–80	=	2
79–75	=	2	79–70	=	3
74–70	=	2			100
69–65	=	1	Average efficiency = 97.7 per cent		
64–60	=	2			
59–50	=	1			
49–35	=	1			
34–20	=	1			
		100			
Average efficiency = 91.4 per cent					

indicated that the absolute temperature may have been reached. The last set of broth tubes showing a positive growth were then used to inoculate other broth tubes. They were heated to the temperature that had previously proved fatal. If the cultures were killed after several trials at a given temperature, it was concluded that the absolute temperature of the strain had been reached.

DISCUSSION OF RESULTS

Table 1 shows the efficiency of pasteurization by comparing the percentages of reduction of the total counts and coli counts

before and after heating the milk. It is noted from the data that considerable reduction in both the total and the coli counts were obtained in a majority of the trials. When these reductions were averaged an efficiency of 91.4 per cent was obtained for the total count and 97.7 per cent for the coli count. Despite the

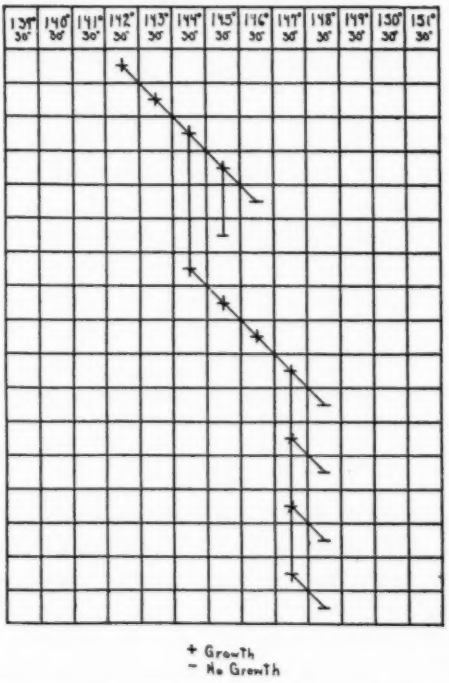

FIG. 1. EFFORTS TO RAISE STRAIN A ABOVE ITS NORMAL THERMAL DEATH-POINT OF 144°F. FOR THIRTY MINUTES

fact that the pasteurization temperature should be sufficient to kill the organisms of the Escherichia-Aerobacter group, we note that a certain percentage of them survived. This occurred in 32 per cent of the number of samples examined.

The survival of Escherichia-Aerobacter organisms in pasteurized milk, as shown in table 1, may be influenced by acclimatiza-

tion. Figure 1 is a graph showing data obtained in raising a strain of *Escherichia coli* above its majority death-point of 144°F. By subculturing those organisms that resisted the various temperatures, it was possible to obtain a strain capable of resisting 147°F, a difference of 3°. These results do not necessarily indicate that this strain has actually acclimatized itself to the higher temperature as it was probably a survival of the fittest. The absolute temperature of a small percentage of the organisms was evidently higher than the majority temperature. This may have resulted in the survival of the most resistant ones. We should, therefore, expect the survival of those bacteria having an absolute death-point above the pasteurizing temperatures.

Reviewing the experimental evidence and the possible biological factors present in pasteurization, the statement can be made that organisms of the Escherichia-Aerobacter group may survive in milk that has been properly pasteurized. Therefore, the coli test cannot be used as a true index of proper pasteurization. This statement opposes the findings of Swenarton (9) and Jenkins (6), but confirms those of De Jong and De Graff (7), Ayres and Johnson (2), Shippen (8), and Hammer (5).

CONCLUSIONS

1. The examination of 100 samples of pasteurized milk showed, in 32 per cent of the samples, that Escherichia-Aerobacter organisms were able to survive the temperatures used in commercial pasteurization.

2. The usual or majority death-point of a culture of *Escherichia coli* found to be 144°F. for thirty minutes, may have its absolute death-point of 145° for thirty minutes raised to 148°F. for thirty minutes. Inasmuch as this latter temperature is above the pasteurization temperature, the survival of members of the coli group may be expected.

3. The experimental evidence herewith presented indicates that the coli test is not a true index to proper pasteurization.

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MINERAL SUPPLEMENTS FOR DAIRY COWS*

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INTRODUCTION

This paper constitutes a final report on six and one-half years' investigation on the feeding of mineral supplements to dairy cows. Three progress reports[1] have already appeared and to these the reader is referred for our conclusions in detail up to the time these reports were published. The results thus far reported were obtained from a study of the feeding of tricalcium phosphate in the form of steamed bone meal specially prepared for animal feeding. In brief, the conclusion reached was that the benefit received from adding steamed bone meal to the ration of dairy cows under conditions existent in New England is very slight.

For the past two years the mineral supplement fed has consisted of a mixture of dicalcium phosphate and calcium carbonate in the respective proportions of four to one.

Köhler and Honcamp[2] have shown that lambs and goats assimilate dicalcic phosphate considerably better than they do tricalcic phosphate in the form of steamed or calcined bone. Having fed steamed bone to our cows for a considerable length of time with largely negative results, it was thought worth while to give the dicalcic phosphate a trial. The only variation from the experimental procedure described in our previous reports other than to substitute the dicalcic phosphate for the tricalcic has been

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[1] The value of calcium phosphate as a supplement to the ration of dairy cows. Jour. Agr. Research., vol. xxxi, pp. 771–791, October 15, 1925.

Mineral matter for dairy cows. Mass. Agr. Expt. Station Bulletin No. 230, April, 1926.

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[2] Landw. Vers. Sta., Bd. LXI, pp. 451–479; *ibid.*, Bd. LXV, pp. 349–380.

a further impoverishment of the ash content of the basal ration. This has been done by substituting for a portion of the hay larger amounts of dried apple pomace than were fed in the previous experiment. The maximum amount of pomace fed to any one cow daily has been seven pounds. According to Kellner's standard[3] the average deficiency of calcium in the basal ration has been about 31 per cent. This is best illustrated by such a concrete example as appears below:

Cow "x" (average of the herd)

	DAILY REQUIREMENT ACCORDING TO KELLNER'S STANDARD	
	Calcium	Phosphorus
	grams	grams
For maintenance (weight 1,115 pounds)	36.2	11.1
For milk (34 pounds)	27.2	17.0
Total required	63.4	28.1
	DAILY INTAKE	
Average ration fed:		
Mixed hay, 17 pounds	37.0	16.2
Dried apple pomace, 5½ pounds	3.8	2.8
Grain mixture, 11½ pounds	3.1	28.2
Total fed	43.9	47.2
Per cent excess or deficit	30.8−	68.0+
Add 8 ounces mineral mixture	61.7	29.9
	105.6	77.1
Per cent excess when mineral was fed	66.6%	174.4%

EXPERIMENTAL METHODS

At the commencement of the experiment the herd consisted of 10 milking Holstein cows, 2 milking Jersey cows, 3 Holstein heifers and 1 Jersey heifer. At the conclusion, due to unavoidable changes, the herd was made up of 10 milking Holsteins and

[3] Kellner's standard calls for a minimum of 32.5 grams of calcium (Ca) and 10 grams of phosphorus (P) daily for maintenance of a 1000-pound cow, and in addition 0.8 gram of calcium and 0.5 gram of phosphorus daily for each pound of milk produced.

3 milking Jerseys, but no heifers. The animals were well housed and received good care, and excepting in bad weather were turned daily into adjacent sheltered yards for air, exercise and sunlight. The cows were barn fed during the entire year; the young stock were turned to pasture from approximately May 20 to October 15.

The entire herd was fed on a basal ration of first cut hay, dried apple pomace and a grain mixture during the entire period of observation, excepting during the summer months, when, in addition, non-leguminous green material in the form of oats, millet and corn were fed in amounts not exceeding 25 pounds daily to any one animal.

The average amount of hay fed daily to the milking cows was 17 pounds; dried apple pomace, 5½ pounds; grain mixture, 11½ pounds; and green feeds, 23 pounds. Green feed (non-leguminous) was fed for a period of about 3 months each season (July 1 to October 1). When fed it was substituted for a portion of the hay in the proportion of 5 pounds of green feed to 1 pound of hay, thus reducing the average daily ration of hay for those months to approximately 12 pounds. Salt was added to the extent of ¾ pound to each 100 pounds of the grain mixture, and lump salt was always available in boxes in the out-of-door sheds. In addition the so-called mineral cows received daily, mixed with the grain, an average of 8 ounces of the dicalcium phosphate-calcium carbonate mixture. The amount of calcium carbonate fed (1 part in 5 of the mixture) was added to maintain the same ratio between calcium and phosphorus as existed when the tricalcic phosphate was fed. Running water was before the animals at all times. The cows were fed and milked twice daily. The hay, which was grown on the station grounds, was composed of mixed grasses, timothy usually predominating, and generally was of good quality. It averaged 7.58 per cent protein, 0.48 per cent calcium and 0.21 per cent phosphorus. The dried apple pomace contained 5.29 per cent protein, 0.15 per cent calcium and 0.11 per cent phosphorus, while the grain mixture, consisting of 30 per cent ground oats, 15 per cent Diamond gluten meal, 15 per cent cottonseed meal, 20 per cent wheat bran and 20 per cent

corn meal, averaged about 17 per cent digestible protein, 45 per cent digestible carbohydrates, 0.06 per cent calcium, and 0.54 per cent phosphorus.

The apple pomace was weighed into wooden trays daily, moistened, and the grain mixed with it before being fed. The hay and green forage were weighed daily into 4-bushel baskets.

Composite samples of all feeds were taken from each lot purchased. A five-day composite sample of the milk of each cow was taken monthly. All feeds and milk were submitted to chemical analyses.

PRESENTATION AND DISCUSSION OF RESULTS

The effect of the supplement has been judged by the same criteria as those used in measuring the effect of tricalcium phosphate, viz: (1) general appearance and body weight of the cows; (2) growth of young cows and heifers; (3) milk yield; (4) composition of the milk, especially its ash content; (5) reproduction.

General appearance

As in all our work on mineral supplements, the general condition of the cows has been followed closely, observations being recorded from month to month. With the exception of one individual the cows in both groups have maintained themselves well. A study of the detailed record of their condition at the commencement of the experiment and again at the close shows that the cows receiving the supplement had a slight advantage in this respect (see table 1).

Body weight

Respecting maintenance of body weight there are six individuals that were mature when the experiment was commenced and which consequently can be considered from this standpoint. The stage of gestation they were in at the beginning having been noted, the date during the present season when they were at a similar stage has been ascertained and the average of two weighings on consecutive days as near that date as possible has been taken (see table 2).

The mature cows receiving the supplement made a considerable gain in weight during the two years of the experiment, while the other group showed little change. The supplement may have made this difference, but with this number of animals it is unsafe to draw a positive conclusion.

TABLE 1

Showing condition of cows

COW NUMBER	AGE JULY 1, 1928	BREED	GENERAL CONDITION	
			July 1, 1926	July 1, 1928
Non-mineral group				
6	11	Grade Holstein	Good	Fair
10	13*	Grade Holstein	Fair	Good*
15	9	Grade Holstein	Very good	Good
35	7	Grade Holstein	Good	Good
42	5†	Grade Holstein	Excellent	Good†
43	6	Purebred Jersey	Good	Excellent
88	3	Grade Holstein	Excellent	Very good
99	3	Grade Holstein	Fair	Fair
Mineral group				
11	13*	Grade Holstein	Good	Fair*
12	11	Grade Holstein	Excellent	Excellent
22	8	Grade Jersey	Fair	Good
33	7	Grade Holstein	Good	Excellent
40	6	Grade Holstein	Good	Good
71	4	Grade Holstein	Fair	Good
98	3	Grade Holstein	Good	Fair
100	3	Grade Jersey	Good	Excellent

* When sold August 8, 1927.

† When slaughtered January 10, 1928.

Growth of young cows and heifers

These animals all received a basal ration low in mineral matter from the time they were weaned at four to five months of age. In addition the "mineral" group received steamed bone meal until the beginning of the present experiment when the dicalcium phosphate-calcium carbonate mixture was substituted for the bone meal.

Ten of the individuals were immature at the commencement of the experiment, six of them being young cows (under five years of age), and four were unbred heifers. In the case of the young cows variations due to variable stage in gestation have been taken into account as with the mature cows. With the heifers unbred when the experiment started obviously this method could not be used, but three of the four are in an almost identical stage of gestation this season and the fourth is also bred, though not

TABLE 2

Maintenance of body weight in the mature cows

COW NUMBER	WEIGHT ON JULY 1, 1926	WEIGHT ON A COMPARABLE DATE THIS SEASON (1928)	GAIN OR LOSS
Non-mineral group			
6	1,270	1,300	+30
10	1,205	1,170*	−35
15	1,315	1,345	+30
Net gain or loss for the group.....			+25
Mineral group			
11	1,275	1,300*	+25
12	1,405	1,510	+105
22	700	820	+120
Net gain or loss for the group.....			+250

* Weight when sold in August, 1927.

as far advanced, so from that standpoint their weights can be considered as quite comparable (see table 3).

The presence of one Jersey in each age class (no. 43 in the young cow class, no. 100 in the heifer class) somewhat complicates analysis of these weight data. A truer picture of the case is obtained if the growth increments are presented on a percentage basis, as in the last column of table 3.

The above weights indicate a considerable difference in favor of the "mineral" group, but it is discounted by the fact that they average six months younger in the case of the young cows and

two months younger in the case of the heifers than the "non-mineral" group. Unfortunately the weights of the groups at identical ages are not comparable due to considerable differences in stage of gestation. If nos. 43 and 71 be dropped out of the

TABLE 3

Growth of young cows and heifers

	WEIGHT ON JULY 1, 1926	WEIGHT ON A COMPARABLE DATE THIS SEASON (1928)	GAIN	
Young cows				
	pounds	*pounds*	*pounds*	*per cent*
Non-mineral group:				
Cow 35	910	1,010	100	11.0
Cow 42	1,260	1,350*	90	7.1
Cow 43	830	850	20	2.4
Net gain for the group			210	
Mineral group:				
Cow 33	1,215	1,355	140	11.5
Cow 40	900	980	80	8.9
Cow 71	955	1,345	390	40.8
Net gain for the group			610	
Heifers				
		WEIGHT ON JULY 1, 1928		
Non-mineral group:				
Heifer 88	685	1,135†	450	65.7
Heifer 99	570	1,140	570	100.0
Total gain			1,020	
Mineral group:				
Heifer 98	575	1,130	555	96.5
Heifer 100	445	855	410	92.1
Total gain			965	

* Weight in October, 1927, no later weight available—cow in isolation stall.

† In a somewhat earlier stage of gestation than the other three heifers.

young cow group the others are of identical age, and the difference is seen to be very small, 9.05 per cent increase in the non-mineral group as against 10.2 per cent in the "mineral" group. Among the heifers if no. 88 were of the same age as the other three (four

months younger than she actually is) her rate of increase in weight would in all probability be enough higher to offset the difference of 11.4 per cent actually found. All things considered the growth of the young animals was probably not materially influenced by the addition of the mineral supplement.

Another angle from which growth records have been viewed is that of weight of the young stock born and reared during the course of the experiment and now in the herd, as compared with the weight of their dams at a similar age. Of the ten individuals listed in table 3 two are as large as their dams were at the same age, six are larger, and two are smaller. The grouping in the experiment is as follows:

Mineral supplement		*No mineral supplement*	
Larger than dam	3	Larger than dam	3
Same size	1	Same size	1
Smaller	1	Smaller	1

All are Holsteins and sired by the same bull, except the two which are the same size as their dams, which are Jerseys. These facts dispose of possible variation due to influence of different sires, and the identical make-up of the two groups is further evidence that the mineral supplement had little, if any, effect on growth.

Photographs of all cows in the herd at the conclusion of the experiment appear in figures 1 and 2.

Milk yield

The average production of the herd has been well maintained all through the experiment. The corrected[4] daily yield per cow for the whole herd has been as follows:

	Milk *pounds*	Total solids *pounds*	Fat *pounds*
First year (10 cows)	26.47	3.32	1.07
Second year (11 cows)	27.59	3.46	1.09

[4] Corrections for differences in length of lactation and stage of gestation have been made according to the method of Gaines and Davidson, described in Illinois Agr. Expt. Sta. Bulletin No. 272. Corrections for age have not been necessary as the groups were of approximately the same age, and the age of the herd as a whole did not increase, as some older cows were sold and heifers took their places. Corrections for breed are taken care of by including in the corrected summary the total solids and fat produced as well as the total milk yield.

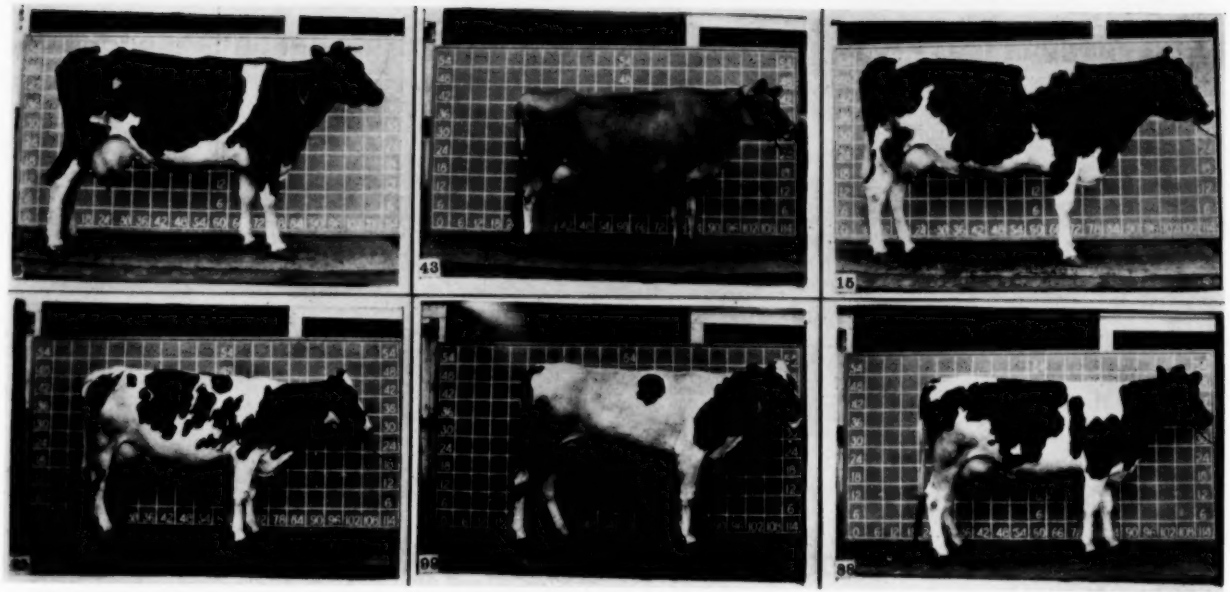

FIG. 1. COWS TYPICAL OF THOSE FED NO MINERALS; TAKEN AT THE CONCLUSION OF THE EXPERIMENT

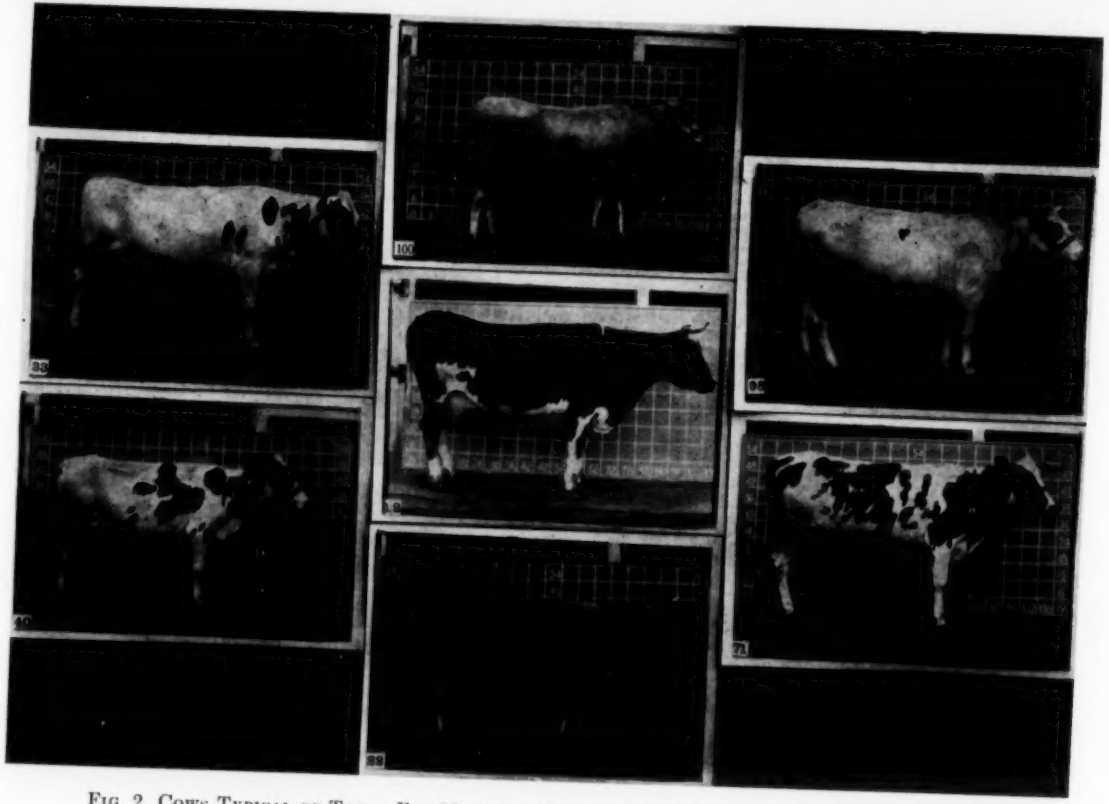

FIG. 2. COWS TYPICAL OF THOSE FED MINERALS; TAKEN AT THE CONCLUSION OF THE EXPERIMENT

The corrected daily yield per cow for the two groups during the entire experiment and year by year appears below:

	Milk *pounds*	Total solids *pounds*	Fat *pounds*
Entire experiment:			
"Mineral" group (10 cow years)	25.09	3.29	1.04
"Non-mineral" group (11 cow years)	28.85	3.58	1.14
Year by Year:			
"Mineral" group (first year, 5 cows)	25.03	3.14	1.00
"Mineral" group (second year, 5 cows)	25.38	3.25	1.02
"Non-mineral" group (first year, 5 cows)	27.92	3.49	1.14
"Non-mineral" group (second year, 6 cows)	29.64	3.65	1.15

TABLE 4

*Composition of the milk**†

	WHOLE HERD	MINERAL GROUP	NON-MINERAL GROUP
Total solids	12.53	12.58	12.53
Fat	3.99	3.96	4.03
Total ash	0.711	0.712	0.710
Ash as per cent of total solids	5.67	5.66	5.66
Calcium	0.121	0.123	0.120
Phosphorus	0.099	0.102	0.097

* Expressed as percentage of the liquid milk.

† These figures have been corrected for differences in the breed make-up of the groups. Corrections for stage of lactation have not been necessary, as the average stage of lactation at the time the samples were taken was the same in both groups.

The "non-mineral" group was somewhat superior to the "mineral" group in production but it would be unwise to conclude from this that the mineral supplement had a detrimental effect in this respect. The probability is that the difference is due to variations in the inherent capacity of the individuals in the two groups to produce milk. An endeavor has always been made to keep the groups as evenly matched in this respect as possible, but due to the impossibility of predicting in advance the performance of a cow, or more especially of a heifer, in any given year, this is a matter which in practice is difficult to regulate. That the endeavor in this particular instance was not successful is evidenced by the figures.

The conclusions that seem justified are two: (1) that the low ash ration apparently had no immediate adverse effect on production; (2) that the mineral supplement apparently had no immediate favorable effect.

Composition of the milk

The milk from each cow in the herd is sampled for five consecutive days each full month that she is in milk. Total solids and fat have been determined each month during the course of the experiment. Total ash, calcium and phosphorus have been determined every three months.

The differences between the groups in this respect are seen to be very slight, and while they favor the mineral group (except in the fat content) they are not of sufficient magnitude to be of any significance, as shown by table 4.

Reproduction

Reproductive troubles have not been serious in either group. The average length of time after calving before reappearance of oestrum has been:

	days
Mineral group	38
Non-mineral group	45

Regarding regularity in recurrence of oestrum the records show the following:

	Good	*Fair*
Mineral group	11	1
Non-mineral group	9	3

The average number of services required the breed has been:

	times
Mineral group	1.13
Non-mineral group	1.30

Seventy-one per cent of the breedings have been from one service, only one cow in each group giving serious trouble in this respect.

Other irregularities have been:

One premature birth (eight months) in the mineral group.

One case of *nymphomania* (chronic buller) in the mineral group.

One case of *cervicitis* (inflammation of the neck of the uterus) in each group.

Three cases of retained afterbirth (two of them in the same cow in successive years) in the non-mineral group.

One calf died *in utero* at about the sixth or seventh month stage as a result of cervical and general uterine infection in the dam. This was in the non-mineral group.

Apparently the cows that received the mineral supplement were a little closer to normal in their reproductive function than those that did not receive it. They came in heat a little sooner after calving, recurrence of heat was slightly more regular, and they bred a little more readily. Other irregularities are about evenly divided between the two groups. In any case the reproductive troubles have not been greater than are ordinarily encountered in practical herd management.

The condition of the calves at birth and subsequently is also of interest in this connection. Twenty-six calves have been dropped in the herd during the course of the experiment. Classification of these according to group and condition appears below.

	Vigorous	*Good*	*Fair*	*Delicate*
Mineral group	3	9	1	1
Non-mineral group	3	5	4	0

The main difference here is seen to be between the "good" and "fair" calves. The statistics undoubtedly favor the mineral group but they need to be examined as to make-up of the groups with respect to sex and breed, as these determine to a considerable extent the apparent vigor of a calf. Bulls are usually more lusty than heifers; Holsteins are generally more rugged than Jerseys.

Of the four "fair" calves in the non-mineral group three were heifers, and the fourth although a bull was a Jersey. On the other hand, of the nine "good" calves in the mineral group, six were heifers, and three of these heifers were Jerseys. These situations just about offset each other so that the balance is still somewhat in favor of the mineral group. It would seem that the cows receiving the mineral supplement produced on the whole somewhat better calves. It must be stated, however, that some

very fine calves have come from the cows that have not received the supplement.

SUMMARY AND CONCLUSIONS

The object of this work has been to determine the value for dairy cows of a mineral supplement consisting of 80 per cent dicalcic phosphate and 20 per cent carbonate of lime added to a ration supposedly deficient in lime. Previous work with tricalcic phosphate in the form of steamed bone meal as a source of lime and phosphorus showed little, if any, advantage in supplying these elements in that form. As dicalcic phosphate is somewhat more soluble and had given better results elsewhere with lambs and goats that the tricalcic phosphate did, it was thought worthy of a trial with cows.

A special effort was made to have the basal ration of the cows low in lime. To this basal ration there was added for half of the herd (known as the "mineral" group) sufficient of the phosphate-carbonate mixture to theoretically make good the deficiency. The experiment was carried on for two years with the following results:

1. With one exception all the cows in the herd maintained their general condition well. The mature cows that received the mineral supplement maintained themselves slightly better in this respect than did those not receiving the supplement. They also made a considerable gain in weight while the non-mineral group showed little change.

2. The supplement had little, if any, effect on the growth of the young cows and heifers.

3. The low ash ration apparently had no immediate adverse effect on milk production and the mineral supplement apparently had no immediate favorable effect.

4. The composition of the milk was not significantly affected.

5. Reproductive troubles were not serious in either group but the cows receiving the mineral supplement were a little nearer normal and produced on the whole somewhat better calves than did those that did not receive it.

6. It must be emphasized that none of the differences between

the groups were sufficiently striking to warrant as a general recommendation the use of the dicalcic phosphate-calcium carbonate mixture. Where cows are average producers (5000 to 8000 pounds) and where they are fed normally on good quality roughage the need of a mineral supplement is not indicated. For heavy producers (10,000 and upwards) it is probably good insurance to supply supplemental lime and phosphorus, but the efficacy of such a practice is by no means well established. It has been nothing short of amazing to note the persistency with which the herd of cows devoted to this study have maintained their milk production and for the most part their own well-being on low ash rations over a period of years. If these cows which average over 9000 pounds of milk yearly, several of them being eleven to twelve thousand pound cows, can make such a showing on abnormal rations, it seems reasonable to infer that the average New England cow on good quality roughage must have a considerable margin of safety as far as minerals are concerned.

This paper is presented with full realization of the beneficial effects reported by numerous other investigators who have fed mineral supplements and without prejudice to their results. Our negative results are attributed in the case of calcium to a considerably higher content of this element in the hays fed than has been found in similar hays elsewhere (0.4 to 0.5 per cent as contrasted with 0.2 to 0.3 per cent), and in the case of phosphorus to quite liberal grain feeding. This latter is normal for New England, and analyses of a large number of Massachusetts hays in addition to those we have fed shows an average calcium content of 0.41 per cent.

It is our opinion that the problem of mineral deficiency in rations is largely a regional one, and that where the roughage carries a reasonably high percentage of calcium and liberal grain feeding is practiced mineral supplements will not be necessary for the average cow.

Regarding Kellner's standard it would seem with respect to calcium that his minimum is well above the danger line, and with respect to phosphorus that where cows are grain fed the amounts of this element they receive are well above the minimum he prescribes.

THE INFLUENCE OF MOLASSES AND SODIUM CHLORIDE IN DAIRY RATIONS ON THE LACTOSE AND CHLORIDE CONTENT AND TASTE OF MILK*

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The importance of taste of dairy products is being emphasized more and more by dairy research workers, commercial distributors of dairy products and by dairy control officers. A knowledge of the factors that influence taste is necessary before progress can be made in improving taste.

Roadhouse and Koestler (1), working with milk at the Dairy and Bacteriology Experiment Station, Liebefeld, Switzerland, found that the chlorine-lactose number[1] of milk as described by Koestler (2) was the greatest single factor in determining the primary taste of the milk. Normal milk that was relatively high in lactose and relatively low in chloride content usually gave a sweet pleasing taste, while milk low in lactose and high in chloride generally gave an unpleasant and often an astringent or salty taste.

In the present experiment an attempt was made to change the lactose-chloride relation of the milk by changing the rations of dairy cows and thus change the taste of the milk.

Morgen, Berger and Fingerling (3) have reported that the concentration of lactose in the milk was not significantly affected by any of the changes in rations which they used. In the present study the maximum amount of carbohydrate, in the form of cane molasses, and the maximum sodium chloride which the cow would consume were fed.

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[1] The value of the chlorine-lactose number is found by multiplying by 100 the ratio of percentage chloride to percentage lactose.

PROCEDURE

Four cows producing milk with a pleasing taste were selected from the Station herd. They were fed a special salt-free basal ration to which no salt was added. No feed was eaten by the animals during the five-hour period previous to milking.

The animals were kept on the basal ration for 26 days and composite samples of milk were collected from twenty-four-hour samples on two consecutive days each week. The samples were examined for taste and for lactose and chloride content. The lactose determinations were made gravimetrically, according to official methods, using Soxhlet and Wein's (4) tables and the chloride determinations by a modification of Volhard's (5) method.

After the preliminary feeding period the same basal ration was continued, and in addition molasses and sodium chloride were given to certain cows for forty-three days, according to the following plan.

Cow 226: Control animal; received the basal ration (without salt).

Cow 404: Received basal ration with a daily average of 2.5 ounces of salt.

Cow 126: Received basal ration with a daily average of 5 pounds of molasses and no salt.

Cow 393: Received basal ration with a daily average of 3.6 ounces of salt and 6 pounds of molasses.

The basal ration consisted of the following:

	Parts
Rolled Barley	4
Oats	2
Wheat bran	2
Linseed meal	1

The sampling and analyses were continued as during the preliminary period. All the cows were then returned to the basal ration for 20 days and the milk sampled and analyzed as previously.

The tables shown in this paper include the averages of the weights of milk, analyses and taste scores of the two consecutive twenty-four-hour samples collected each week during the course of the experiment.

TABLE 1

Cow 226. Lactose and chloride content and taste score of milk from control cow receiving basal ration (without salt)

	DATE	MILK 24 HOURS	TASTE SCORE	PER CENT LACTOSE	CHLORIDE
	1919	*pounds*			*grams per liter*
Preliminary period	March 8	32.7	13.52	4.82	0.83
	March 12	31.7	13.48	4.81	0.87
	March 20	32.3	13.54	4.76	0.93
	March 26	34.0	13.50	4.78	0.96
	April 2	33.7	13.32	4.69	0.97
Experimental period	April 10	31.8	13.27	4.81	0.95
	April 16	32.9	13.31	4.67	0.96
	April 24	35.1	13.34	4.71	0.94
	May 1	33.7	13.31	4.73	0.94
	May 8	32.5	13.39	4.74	0.97
	May 15	32.9	13.31	4.63	0.99
Post-experimental period	May 22	32.3	13.35	4.66	1.00
	May 29	28.3	13.28	4.72	1.08
	June 4	27.9	13.45	4.74	1.04

TABLE 2

Cow 404. Lactose and chloride content and taste score of milk from cow receiving basal ration and sodium chloride

	DATE	MILK 24 HOURS	TASTE SCORE	PER CENT LACTOSE	CHLORIDE
	1919	*pounds*			*grams per liter*
Preliminary period	March 8	37.7	13.57	4.84	0.77
	March 12	34.2	13.56	5.09	0.71
	March 17	34.5	13.55	5.03	0.71
	March 20	33.1	13.47	5.06	0.76
	March 26	32.5	13.48	4.93	0.79
	April 2	32.1	13.52	5.03	0.72
Experimental period	April 10	29.1	13.16	5.00	0.82
	April 17	29.8	13.46	4.74	0.85
	April 24	28.7	13.42	4.64	0.90
	May 1	29.0	13.44	4.73	0.82
	May 8	26.2	13.27	4.86	0.81
	May 15	27.7	13.38	4.71	0.83
Post-experimental period	May 22	28.1	13.40	4.76	0.84
	May 29	28.4	13.38	4.84	0.84
	June 4	27.6	13.50	4.80	0.85

TABLE 3

Cow 126. Lactose and chloride content and taste score of milk from cow receiving basal ration and molasses

	DATE	MILK 24 HOURS	TASTE SCORE	PER CENT LACTOSE	CHLORIDE
	1929	*pounds*			*grams per liter*
Preliminary period	March 8	19.5	13.36	4.50	1.24
	March 12	20.5	13.37	4.45	1.32
	March 20	19.3	13.38	4.47	1.34
	March 26	21.1	13.34	4.46	1.34
	April 2	20.3	13.38	4.42	1.35
Experimental period	April 10	20.2	13.40	4.43	1.36
	April 16	20.2	13.38	4.35	1.42
	April 24	21.1	13.16	4.34	1.40
	May 1	20.1	13.25	4.37	1.42
	May 8	18.8	13.32	4.44	1.37
	May 15	17.9	13.23	4.40	1.39
Post-experimental period	May 22	17.2	13.38	4.47	1.39
	May 29	14.5	13.35	4.54	1.44
	June 4	14.1	13.35	4.48	1.43

TABLE 4

Cow 393. Lactose and chloride content and taste score of milk from cow receiving basal ration with molasses and sodium chloride

	DATE	MILK 24 HOURS	TASTE SCORE	PER CENT LACTOSE	CHLORIDE
	1929	*pounds*			*grams per liter*
Preliminary period	March 8	24.6	13.48	4.42	0.90
	March 12	26.3	13.42	4.45	0.90
	March 20	24.5	13.45	4.49	0.92
	March 26	25.8	13.43	4.42	0.94
	April 2	25.6	13.46	4.34	0.97
Experimental period	April 10	20.9	13.49	4.34	0.97
	April 16	21.5	13.48	4.29	1.01
	April 24	23.7	13.47	4.13	0.99
	May 1	22.8	13.47	4.16	1.02
	May 8	20.5	13.45	4.22	1.00
	May 16	20.9	13.42	4.13	1.07
Post-experimental period	May 22	20.0	13.32	4.18	1.10
	May 29	17.5	13.38	4.32	1.13
	June 4	14.4	13.38	4.28	1.08

DATA

The results secured from the feeding, sampling and analyses of each animal are shown in tables 1 to 5, inclusive.

The summary of the data presented in tables 1 to 4 is shown in table 5 and is an average of total analyses for the preliminary, experimental and post-experimental periods.

TABLE 5

The average lactose and chloride contents and taste scores of the milk of each cow during the periods of the experiment

	COW	PRELIMINARY PERIOD	EXPERIMENTAL PERIOD	POST-EXPERIMENTAL PERIOD
Per cent lactose	226	4.77	4.72	4.71
	404	5.00	4.78	4.80
	126	4.46	4.39	4.50
	393	4.42	4.21	4.26
Chloride, grams per liter	226	0.91	0.96	1.04
	404	0.74	0.84	0.84
	126	1.32	1.39	1.42
	393	0.93	1.01	1.10
Taste scores	226	13.51	13.32	13.36
	404	13.53	13.37	13.43
	126	13.37	13.29	13.36
	393	13.45	13.46	13.36

DISCUSSION OF RESULTS

Cow 226, the control, showed a very slight average decrease of 0.06 per cent lactose during the entire period, which is considered normal with the advance in the period of lactation. The chloride increased 0.13 gram per liter during the entire period, which also can be considered normal with the advance of lactation. The taste score approximately paralleled the lactose content.

Cow 404, receiving an average of 2.5 ounces of sodium chloride daily after the preliminary period, produced milk with a very slight average decrease of 0.22 per cent lactose during the experimental period, and 0.2 per cent during the post-experimental period.

The chloride content increased an average of 0.1 gram per liter during the experimental period, which is considered normal with the advance in lactation. It is indicated from these results that the chloride was not abnormally increased in the milk with the increase of chloride in the ration.

The taste score decreased an average of 0.16 of a point during the experimental period, and corresponds closely with the lowering of the lactose content during the same period.

Cow 126, receiving an average of 5 pounds of molasses daily, after the preliminary period produced milk containing an average of 0.07 per cent less lactose during the feeding of the molasses than during the preliminary period, and 0.11 per cent less during the post-experimental period. It is evident from these results that the feeding of the molasses did not increase the lactose content during the experimental feeding.

The chloride content increased 0.1 gram per liter during the experimental period, which is considered normal with the advance in lactation.

The taste score averaged 0.08 of a point lower during the experimental period, which would be expected to accompany the lower lactose content.

Cow 393, receiving an average of 6 pounds of molasses and 3.6 ounces of salt daily after the preliminary period, produced milk containing 0.21 per cent less lactose during the experimental period than during the preliminary period, and 0.05 per cent less than during the post-experimental period. The results indicate that the molasses also fed to this cow did not increase the lactose content during the experimental feeding.

The chloride content increased slightly and at the same rate during the preliminary, experimental and post-experimental periods, and at approximately the same rate as the control cow. This increase in chloride content would be expected during the advance of lactation.

The taste score remained constant during the preliminary and experimental periods, with a slight decrease of an average of 0.1 point during the post-experimental period.

The results indicate that the feeding of molasses and sodium

chloride did not increase the lactose or abnormally increase the chloride content in the milk.

CONCLUSIONS

1. The addition of molasses in the amount of 5 to 6 pounds daily to a basal ration of grain and alfalfa hay, fed to two cows, did not increase the lactose content of the milk.

2. The addition of sodium chloride in the amount of 2.5 to 3.6 ounces daily to a basal ration of grain and alfalfa hay, fed to two cows, did not increase the chloride content of the milk abnormally.

3. In general, the taste score of the milk followed the trend of the lactose content; the taste score lowering and rising as the lactose per cent decreased and increased. It is also to be noted that there was a correlation between the lowering of the taste score and lactose percentage and an increase in the chloride content.

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FACTORS INFLUENCING THE PROPERTIES OF FERMENTED RECONSTRUCTED MILK*

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INTRODUCTION

The limited supply of normal skim milk suitable for fermented milk manufacture, especially in the large centers of population, together with the steadily growing demand for fermented milks, has caused the plant operators to search for a substitute for normal skim milk.

This paper presents the results of a study of the influence of the manufacturing procedure and of the quality of starter used, on the fermented milk made from reconstructed skim milk. The processes studied are those that are subject to considerable variations between plants, or in the same plant.

REVIEW OF LITERATURE

Burke (1) defined the qualities of a good grade of Commercial Buttermilk.

Larsen and White (2) found that a good quality starter can be made by using reconstructed skim milk as a substitute for normal skim milk. They further showed the danger of using too much powder in the manufacture of the reconstructed milk.

Dahle and Palmer (3) in an investigation of the properties of reconstructed milks made a milk which gave a slightly lower soluble protein content, a slightly lower buffer action at low concentrations of alkali and acid, had a slightly higher viscosity than normal milk, but closely resembled normal milk in freezing point, specific gravity, and specific conductivity.

Hammer (4) recommended an incubation temperature of from 21 to 23°C. (70° to 74°F.) for the commercial cultures.

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Knaysi (5) studied fermented normal milk and found that an increase in the amount of inoculum gave an increase in titratable acidity, viscosity, firmness of the curd, and an increased tendency to whey off.

Hammer (6) and Knaysi (5) concur in their findings that an increase in the pasteurization exposures increases the firmness of the curd of fermented normal milk.

Hammer and Baker (7) found that lowering the pasteurization exposures lowered the rate of acidity development and lengthened the time of coagulation.

Yaxis (8), Messcher (9), and Burke (10), and Reid (11) agree that the addition of small amounts of gelatin to normal skim milk before fermentation will result in an increased viscosity and prevents wheying off of the fermented milk.

PROCEDURE

Studies were made of the effect of the following factors on the fermented milk made from reconstructed skim milk:

1. The quantity of inoculum.
2. The process of cooling after incubation.
3. The pasteurization exposure.
4. The addition of different amounts of gelatin.
5. The addition of normal skim milk.
6. The cooling of fermented milk before breaking the curd.
7. A comparison of the qualities of fermented milks made from gelatinated reconstructed skim milk, normal skim milk, and reconstructed skim milk.

Reconstructed skim milk was made for these studies by the addition of water to skim milk powder in the ratio of 9 pounds of water to 1 pound of skim milk powder. The skim milk powder used for these studies was selected by giving careful consideration to its aroma, flavor, solubility and acidity.

The milk so made was then pasteurized at 180°F. for thirty minutes, cooled to 72°F., transferred into sterile quart bottles, and inoculated.

The inoculated milk was then incubated at a temperature of

72°F. for a period of from twelve to fourteen hours and then removed to a cooling room, the temperature of which was maintained at 42°F., for a period of eight hours.

After cooling the curd was broken by gently agitating the bottles, each bottle receiving a like amount of agitation.

Three samples were then transferred into sterile bottles for testing and scoring at the end of twenty-four, seventy-two, and one hundred and twenty hours storage at 42°F. The remainder of each lot was used for immediate testing and scoring.

The aroma and flavor of each lot were scored on the basis of 100 points and a score of 75 for aroma and flavor was deemed palatable. The body and texture were scored on the basis of 100 points and a score of 85 was deemed necessary to be considered a good quality of fermented milk. The acidity was determined by titration against tenth normal sodium hydroxide, and calculated as per cent lactic acid. The viscosity time was determined by the time in seconds required to empty a 50 cc. pipette to a mark 1 inch below the bulb. The whey formation, the appearance of the curd, and the presence of gas were also noted.

EXPERIMENTAL DATA

The inoculation of reconstructed milk with various percentages of starter

Six series, of 5 lots each, were made to determine the effect of varying the amount of starter used in the inoculation.

Table 1 shows the effect of inoculating the reconstructed milk with increasing amounts of starter. Increasing the increment of inoculum increased the score of the aroma, flavor, body, texture, percentage of acidity, and the viscosity in the fermented milks, at the time of breaking the curd. The increase in the score of the aroma and flavor was largely the result of a submerging of powder taste and odor due to the increased acidity. The increase in body and texture score was due to the development of a stronger, more viscous curd, all lots showing a consistent smoothness.

The appearance of the respective lots was not affected and no wheying off occurred in the fresh buttermilk.

Storage for twenty-four hours resulted in a decrease in the score of the aroma, flavor, body, and texture, in titratable acidity, and in viscosity time, followed by a slight increase at the end of seventy-two hours storage. Continued storage at the same tem-

TABLE 1

The effect on the properties of fermented reconstructed milk when different percentages of starter were added

NUMBER OF LOT	INOCULUM ADDED	SCORE OF AROMA-FLAVOR	SCORE OF BODY-TEXTURE	ACIDITY	VISCOSITY TIME
	per cent	*per cent*	*per cent*	*per cent*	*seconds*
I	2	75.60	79.00	0.789	59
II	4	77.80	80.50	0.807	72
III	6	78.80	83.00	0.853	72
IV	8	78.80	84.00	0.855	77
V	10	79.60	84.30	0.863	78

TABLE 2

The effect on the whey formation of fermented reconstructed milk when different percentages of starter were added

NUMBER OF LOT	INOCULUM ADDED	SAMPLES WHEYING OFF AND AMOUNT OF WHEY FORMED ON STORAGE					
		24 hours		72 hours		120 hours	
		⅛–¼ inches	¼–½ inches	⅛–¼ inches	¼–½ inches	⅛–¼ inches	¼–½ inches
	per cent						
I	2	1	0	2	0	2	0
II	4	1	1	2	1	2	1
III	6	0	2	0	1	0	1
IV	8	0	1	0	0	0	1
V	10	0	2	0	0	0	1

perature caused a downward trend in the numerical values of the properties at the end of one hundred and twenty hours.

Table 2 shows the whey formation of the fermented milk. The results show a tendency for lots I and II to whey off on storage. A slight amount of whey, however, was not considered harmful to the product.

The appearance of the samples, excepting those showing considerable whey formation, was very uniform.

The effect of the cooling process after pasteurization on the properties of fermented reconstructed milk

Six series, of 4 lots each, were made to determine the effect of the cooling process on the properties of the fermented reconstructed milk.

Cooling the heated milk over a surface cooler tends to give a product somewhat less desirable in flavor and aroma as shown in table 3, and is thought to be due to the aeration of the heated product. Quick cooling does not result in any marked improvement in the properties of the reconstructed milk because of the danger of contamination due to the exposure to air. Variations occurring in the numerical value of the other properties are insignificant and show little relationship to the treatment of the different lots.

TABLE 3

The effect of the cooling process after pasteurization on the properties of fermented reconstructed milk

NUMBER OF LOT	COOLING PROCESS		SCORE OF AROMA-FLAVOR	SCORE OF BODY-TEXTURE	ACIDITY	VISCOSITY TIME
	Cooling medium	Temperature after cooled				
		°F.	*per cent*	*per cent*	*per cent*	*seconds*
I	Tap water	72	79.00	77.80	0.897	113
II	Ice water	72	79.30	77.80	0.903	120
III	Surface cooler	72	77.60	78.00	0.901	109
IV	Surface cooler	50	78.00	77.70	0.901	113

Wheying off did not occur in any of the lots other than a very slight amount formed on the surface, and was not considered harmful to the quality of the fermented milk. Cooling by different processes had no effect on the appearance of the samples.

Storage of the samples for a period of five days produced no whey and did not effect the appearance of the samples.

The effect of the pasteurization exposure on the properties of fermented reconstructed milk

Six series, of 4 lots each, were made to determine the effect of the pasteurization exposure on the fermented milk.

Table 4 shows the effect of the pasteurization exposure at the time of breaking the curd. Increasing the pasteurization temperature above 180°F., or exposing the milk for a period exceeding thirty minutes decreased the score of the aroma and flavor by causing a powder taste and odor to become apparent. The cooked aroma and flavor so often appearing in normal milks when heated to a high temperature did not appear in the reconstructed milk.

TABLE 4

The effect of the pasteurization exposure on the properties of fermented reconstructed milk

NUMBER OF LOT	PASTEURIZATION		SCORE OF AROMA-FLAVOR	SCORE OF BODY-TEXTURE	ACIDITY	VISCOSITY TIME
	Temperature	Exposure				
	°F.	minutes	per cent	per cent	per cent	seconds
I	180	30	79.60	84.20	0.859	91
II	180	60	79.00	84.60	0.863	96
III	200	30	77.60	84.20	0.863	103
IV	200	60	74.00	83.80	0.872	127

TABLE 5

The effect of the addition of gelatine on the properties of fermented reconstructed milk

NUMBER OF LOT	GELATINE	SCORE OF AROMA-FLAVOR	SCORE OF BODY-TEXTURE	ACIDITY	VISCOSITY TIME
	per cent	per cent	per cent	per cent	seconds
I	0.00	80.10	86.60	0.890	109
II	0.15	80.80	88.30	0.890	126
III	0.25	80.00	89.60	0.896	149
IV	0.35	81.60	92.50	0.904	206

The score of the body and texture was not materially affected by the different pasteurization temperatures or periods of exposure. A slight increase in acid and a considerable increase in the viscosity resulted with an increase in the time or temperature of the pasteurization exposure.

Increasing the pasteurization exposure imparted a creamy appearance to the fermented milk. Whey formation was very limited and equally distributed among the samples.

There was no apparent relationship between the length of the pasteurization period, and the amount of whey formed or the appearance of the samples.

The effect of the addition of gelatin on the properties of the fermented reconstructed milk

Six series, of 4 lots each, were made to determine the effect of the addition of small amounts of gelatin on the properties of fermented reconstructed milk.

Table 5 shows the improvement of the properties of the fermented milk at the time of breaking the curd. The score of the aroma and flavor was not materially affected by the addition of gelatin. The presence of gelatin in small amounts caused a marked increase in the body and texture score of the samples. With each additional increment of gelatin there was a slight increase in the acidity of the samples. A marked increase in the viscosity is shown with increasing amounts of gelatin. Lot IV was increased almost one hundred per cent in viscosity time over lot I.

Lots III and IV appeared much firmer in curd than lots I and II; Lot IV in two instances was described as having a somewhat jelly-like consistency.

No appreciable amount of whey formed in any of the samples.

The effect of the addition of normal skim milk on the properties of fermented reconstructed milk

Four series, of 4 lots each, were made to determine the effect of the addition of normal skim milk on the properties of the fermented reconstructed milk.

The addition of normal skim milk to reconstructed milk increased the desirability of the fermented milk at the time of breaking the curd, as shown by table 6. The scores of aroma, flavor, body, and texture were constantly higher in those lots to which normal skim milk had been added before fermentation. Normal skim milk seemed to impart a richer aroma and flavor, and a more velvety body and texture to the fermented milk.

The acidity and the viscosity time of the fermented milk were not materially effected by the addition of different volumes of normal skim milk.

Wheying off did not occur in any of the lots and the appearance of all lots was uniform.

The keeping qualities of the fermented milks were much the same. The lots to which normal skim milk had been added, maintained their initial advantage in the scores of aroma, flavor,

TABLE 6

The effect of the addition of normal skim milk on the properties of fermented reconstructed milk

NUMBER OF LOT	AMOUNT OF NORMAL SKIM	SCORE OF AROMA-FLAVOR	SCORE OF BODY-TEXTURE	ACIDITY	VISCOSITY TIME
	per cent	*per cent*	*per cent*	*per cent*	*seconds*
I	None	81.20	89.50	0.890	152
II	10	83.20	91.20	0.892	151
III	20	87.50	94.50	0.893	155
IV	30	88.50	94.50	0.891	153

TABLE 7

The effect of cooling before breaking the curd on the properties of fermented reconstructed milk

NUMBER OF LOT	TIME BEFORE BREAKING CURD	SCORE OF AROMA-FLAVOR	SCORE OF BODY-TEXTURE	ACIDITY	VISCOSITY TIME
	hours	*per cent*	*per cent*	*per cent*	*seconds*
I	0	79.00	82.50	0.867	93
II	4	80.00	83.50	0.862	89
III	6	80.70	84.70	0.870	93
IV	8	81.00	87.00	0.878	91

body, and texture; all lots depreciating in score for twenty-four hours rather rapidly, with a subsequent slower rate of decline in value for the period of five days.

Storing the samples produced no whey and the appearance of the samples of fermented milk remained much the same.

The effect of cooling before breaking the curd on the properties of fermented reconstructed milk

Four series, of 4 lots each, were made to determine the effect of cooling before breaking the curd. All lots were then sampled and tested.

The effect of cooling after incubation, on the properties of the fermented milk is shown in table 7. A slight increase in the scores of aroma, flavor, body, and texture was recorded in those lots stored for longer periods before breaking the curd. The gain in score in every instance increased with the time stored before breaking the curd.

The acidity of the samples held for longer periods of time before breaking the curd increased slightly. The viscosity time, however, was not effected by cooling before breaking the curd.

TABLE 8

The keeping qualities of fermented milk stored at 39°F.

NUMBER OF LOT	STORAGE	SCORE OF AROMA AND FLAVOR	SCORE OF BODY AND TEXTURE	ACIDITY	VISCOSITY TIME
	hours	*per cent*	*per cent*	*per cent*	*seconds*
I	0	84.00	88.00	0.872	158
II	0	83.50	84.50	0.882	118
III	0	83.00	80.50	0.875	104
I	24	86.00	90.00	0.882	208
II	24	86.00	86.00	0.905	142
III	24	85.50	84.20	0.873	106
I	72	88.50	90.00	0.862	268
II	72	88.50	86.00	0.875	216
III	72	87.50	85.00	0.890	114
I	120	85.50	88.50	0.852	248
II	120	87.00	87.50	0.862	157
III	120	84.50	84.50	0.875	140

Only a very slight amount of whey formed in any case, and that formed in the individual samples, showed no relation to the treatment of the curd.

Storage of the samples produced no evidence of whey formation and no change in the appearance of the samples.

A comparison of the properties of fermented milks made from gelatinated reconstructed skim milk, normal skim milk, an reconstructed skim milk at various storage temperatures

The reconstructed skim milks were made by the outlined procedure. The gelatin was added to the skim milk powder

before the addition of water. All milks were made into fermented milk by the same process.

Two series, of 3 lots each, were made to furnish data for comparative purposes. The data given are the averages of the two series.

Lot I was made from reconstructed skim milk to which 0.35 per cent of gelatin, of 175 Bloom test, was added.

Lot II was made from normal skim milk.

Lot III was made from reconstructed skim milk, to which no gelatin was added.

TABLE 9

The keeping qualities of fermented milk stored at 60°F.

NUMBER OF LOT	STORAGE	SCORE OF AROMA AND FLAVOR	SCORE OF BODY AND TEXTURE	ACIDITY	VISCOSITY TIME
	hours	*per cent*	*per cent*	*per cent*	*seconds*
I	0	84.00	88.00	0.872	158
II	0	83.50	84.50	0.882	118
III	0	83.00	80.00	0.875	104
I	24	82.50	85.50	0.907	119
II	24	81.50	82.50	0.930	90
III	24	81.00	81.00	0.912	65
I	72	81.00	83.00	0.925	134
II	72	79.50	77.00	0.935	90
III	72	78.50	75.50	0.910	67
I	120	78.50	83.50	0.922	84
II	120	79.50	75.00	0.932	
III	120	77.00	73.50	0.902	

At the time of breaking the curd 12 samples were taken from each lot. Three samples were stored at 37° to 39°F., 3 at 58° to 60°F., 3 at 72°F., and 3 were stored at room temperature.

Tables 8 to 11, inclusive, show the properties of the fermented milks at the time of breaking the curd and during the storage period of five days.

Storing the samples at 39°F., as shown by table 8, resulted in an increase in the scores of the aroma, flavor, body, and texture during the seventy-two-hour period, followed by a decline in the

scores at the end of the five-day period. Lots I and II showed the same general trend in the viscosity time, while Lot III continued to gain through the five-day period.

TABLE 10

The keeping qualities of fermented milk stored at 72°F.

NUMBER OF LOT	STORAGE	SCORE OF AROMA AND FLAVOR	SCORE OF BODY AND TEXTURE	ACIDITY	VISCOSITY TIME
	hours	*per cent*	*per cent*	*per cent*	*seconds*
I	0	84.00	88.00	0.872	158
II	0	83.50	84.50	0.882	118
III	0	83.00	80.50	0.875	104
I	24	80.00	82.00	0.907	54
II	24	80.00	80.00	0.932	41
III	24	78.50	79.00	0.912	35
I	72	76.50	78.50	0.925	31
II	72	76.50	75.50	0.957	25
III	72	74.00	75.00	0.942	26

TABLE 11

The keeping qualities of fermented milk stored at room temperature

NUMBER OF LOT	STORAGE	SCORE OF AROMA AND FLAVOR	SCORE OF BODY AND TEXTURE	ACIDITY	VISCOSITY TIME
	hours	*per cent*	*per cent*	*per cent*	*seconds*
I	0	84.00	88.00	0.872	158
II	0	83.50	84.50	0.882	118
III	0	83.00	80.50	0.875	104
I	24	82.50	83.00	0.915	80
II	24	82.00	81.00	0.930	63
III	24	79.50	78.50	0.905	46
I	72	79.00	81.50	0.915	55
II	72	79.00	78.50	0.950	38
III	72	74.00	75.00	0.917	33
I	120	75.50	80.00	0.905	57
II	120	75.50	75.50	0.937	32
III	120	72.50	75.00	0.900	25

The acidity of lots I and II developed somewhat during the first twenty-four hours of storage, followed by a decline during

the remainder of the storage period. Lots III continued to gain in acidity for three days, followed by a decline at the end of the fifth day.

Storage of the samples at 60°F., as shown by table 9, resulted in a gradual decline in the scores of the aroma, flavor, body, and texture during the storage period of five days. The acidity increased gradually during the seventy-two-hour period, followed by a decrease at the end of the fifth day. The viscosity of all lots showed a marked decrease at the end of the first 24 hours storage, followed by a tendency to remain constant in the instances of lots II and III, while lot I experienced some fluctuation.

Storage of the samples at 72°F. as shown by table 10, resulted in a very rapid decline in the properties of the fermented reconstructed milk. The score, of aroma, flavor, body, and texture show marked decreases at the end of the twenty-four hours storage, and further decreases at the end of the three-day period.

Storage of the samples at room temperature resulted in a general decline in the aroma, flavor, body, texture, and viscosity time, as shown by table 11; the acidity increasing for three days followed by a decrease at the end of the fifth day. The changes taking place in the samples held at room temperature were much the same as those taking place in the samples held at 60°F.; however, the changes proceeded at a faster rate, particularly during the seventy-two-hour and the one-hundred-and-twenty-hour period.

DISCUSSION OF EXPERIMENTAL DATA

Increasing the amount of inoculum improved the aroma and flavor and increased the acidity of the fermented milk made from reconstructed skim milk. Cordes and Hammer (12) found, in the fermentation of normal skim milk, higher total acidities resulted in the production of a higher percentage of volatile acids by *S. Lacticus* and the organisms associated with it in commercial cultures. The same is no doubt true of fermented reconstructed skim milks. The increased amount of volatile acid present may account for the improvement in aroma and flavor in the fermented milks made from reconstructed skim milk inoculated with higher percentages of starter.

An increase in the amount of inoculum resulted in the improvement in the body and texture of the fermented milk and in a slower rate of deterioration of the body on storage, due to a more complete fermentation. The weakness of the curd due to low acid development would reflect itself in the score of the body and texture, and caused a more rapid deterioration in the body and texture of the fermented milk on storage.

The method used and the rapidity of reducing the temperature of the milk after pasteurization had little effect on the properties of the fermented milk except that the surface cooler method which has an advantage in point of time to cool, and in the aeration of the pasteurized milk, endangers the milk by possible contamination.

Increasing the pasteurization exposure of the milk to be fermented results in an inferior aroma and flavor in the fermented milk. The slight powder taste and odor common to all desiccated milks and present at all times in the fermented milk, is intensified by high temperatures or even moderate temperatures (180°F.), on long exposure, and becomes more apparent in the fermented milk on storage, probably due to the loss of volatile acids.

The addition of small amounts of gelatin to the reconstructed skim milk to be fermented results in an improvement in the body and texture of the fermented milk. The presence of a gel incorporating the curd produces a heavier, more viscous body, which would retard the formation of whey, and cause a slower rate of deterioration of the body.

That the nature of the proteins of milk are changed on dessication was shown by Dahle and Palmer (3). The change in the nature of the proteins reflects itself in the formation of a weaker curd on fermentation as shown by table 6. The addition of normal skim milk in relatively large quantities would cause a more normal fermentation to take place and would account for the formation of a firmer curd with a consequent increase in the score of the body and texture and an improvement of the aroma and flavor.

Cooling the fermented milk before breaking the curd improves the aroma, flavor, body, and texture of the product as shown by

table 7. The added fermentation taking place, as shown by the increased acidity present, would be a large factor in the improvement in the fermented milk due to the presence of more volatile acids, and to the formation of a firmer curd.

A comparison of the properties of fermented milks made from gelatinated reconstructed skim milk, normal skim milk, and reconstructed skim milk to which no gelatin had been added, table 8, shows them to be nearly equal in the scores of aroma and flavor. The apparent ability of *S. Lacticus* to grow better in normal skim milk with the production of more volatile acids probably accounts for the richer aroma and flavor of the fermented milk made from normal skim milk.

The body and texture of the lots made from gelatinated reconstructed skim milk were judged to be superior to the lots made from normal skim milk. The presence of a slight lumpiness decreased the score of the body and texture in those lots made from normal skim milk. The lumpiness of the lots made from the normal skim milk was not considered as objectionable as the weakness of the lots made from reconstructed skim milk to which no gelatin had been added.

Storage of fermented milks at temperatures of 60°F. or above, will result in the rapid deterioration of the product as those temperatures are suitable for the growth and development of different types of bacteria. The development of *S. Lacticus* and associated groups is inhibited by a high acidity development as pointed out by Hammer (6). However, the growth of other organisms that may have escaped the pasteurization exposure is not inhibited and may result in the production of off aromas and flavors and in the disintegration of the body by proteolytic bacteria.

CONCLUSIONS

1. A fermented milk possessing very desirable qualities can be made from reconstructed skim milk when the processes pertinent to its manufacture are recognized and uniformly controlled.

2. Fermented milks made from reconstructed skim milk are characterized by a body and texture that are consistently very smooth.

3. The quality of the fermented milk may be improved or impaired by modifying the manufacturing process.

4. The quality of the starter used is a factor of utmost importance. An amount of high quality starter as practical in commercial practice should be used.

5. The cooling process subsequent to pasteurization has no important effect on the quality of fermented milk made from reconstructed skim milk.

6. Pasteurization of the reconstructed skim milk at high temperatures or for long exposures should be avoided.

7. The addition of 0.35 per cent of gelatin can be recommended as improving the body and texture of the fermented milk made from reconstructed skim milk.

8. The addition of normal skim milk in amounts exceeding 10 per cent can be recommended.

9. Cooling of the fermented milk prior to the breaking of the curd should be practiced whenever possible.

10. Storage of fermented milk made from reconstructed skim milk at a temperature of 39°F. causes no deterioration in the quality of the product in five days.

11. Storage temperatures above 42°F. causes a decrease in quality after three days while a storage temperature of 60°F. results in an inferior product in twenty-four hours.

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α AND β LACTOSE IN SOME MILK PRODUCTS*

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INTRODUCTION

The α hydrate form of lactose has been known for a long time. It is this form which crystallizes from sweetened condensed milk and ice cream, and in the commercial manufacture of milk sugar.

Erdmann (4) in 1855 was the first to call attention to another form of lactose which we now recognize as the β form. It crystallizes from a concentrated solution of lactose at the boiling point. He observed that the optical rotation of a freshly prepared solution of β lactose increased with time while a freshly prepared solution of α lactose decreased with time. Dubrunfant (3) also observed the decrease in rotation of freshly prepared α lactose solutions. Interest in the β form of lactose was revived some time later by Schmoger (21) (22) and Erdmann (5). Erdmann's method of preparing β lactose by evaporating a solution to dryness resulted in a browned impure product. Tanret (25) tried to improve upon Erdmann's method by redissolving the β lactose in water and reprecipitating with alcohol. Tanret and others thought there were three forms of lactose. Hudson (11) (12) studied the solubility of lactose under varying conditions and showed that the third form was a mixture of α and β lactose. Gillis (6) gives phase diagrams for lactose-water systems and also additional information on the solubility of lactose.

Lactose tends to form supersaturated solutions. Attention was called to this behavior as early as 1856 by Debrunfant (3). The supersaturation which can ordinarily be obtained has been shown by Leighton and Peter (17). The effect of sucrose on the solubility of lactose has been studied by Hunziker and Nissen (14)

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and Peter (19). An extensive review of the general literature on lactose has been given by Whittier (30).

A study of lactose has been in progress for some time in this laboratory. Sharp (23) attempted to point out the possible significance of the rate of change of β to α lactose as an important factor in controlling the crystallization of lactose from condensed milk, ice cream and concentrated lactose solutions. This paper is a preliminary report of a phase of this work dealing with the relative amounts of α and β lactose in dairy products, together with a study of some factors causing a change in the relative amounts of the two forms.

EXPERIMENTAL

The effect of the pH of the solution on the rate of conversion of the forms of lactose into each other

The optical rotation of a freshly prepared solution of α lactose is higher than it is after standing for some time, the decrease in rotation being due to the change of some of the α into the β form. The specific rotation of the β form is much lower than is the specific rotation of the α form. The specific rotation of α lactose is 86.0° and of β lactose 35.4° at 20°C. according to Hudson (12). Erdmann (4) and Urech (27) observed that acids accelerated the rate of decrease in rotation of α lactose solutions and Urech observed that ammonium hydroxide caused the change to be almost instantaneous. Trey (26) investigated the effect of a number of substances as influencing the rate of change of rotation of lactose solutions. He clearly showed that alkalies accelerate the rate of change more than do acids and if too much alkali is added decomposition begins. Bleyer and Schmidt (1) stated that very strong acid solutions displaced the equilibrium toward the formation of α lactose while strong alkalies displaced it toward the formation of β lactose.

It seems that these various substances for the most part exert their accelerating effect on the rate of change of the forms of lactose into each other by a change in the hydrogen ion concentration, therefore, the influence of pH upon this behavior was determined.

The change of the two forms of lactose into each other can be followed quite conveniently by means of the polariscope. Urech (28) called attention to the monomolecular character of this reaction. It is an incomplete reaction which comes to equilibrium when there is present in the solution about 1.58 parts of β to 1.0 part of α lactose at room temperature. The rate of change is given by the following equation:

$$\frac{dx}{dt} = k_1 (a - x) - k_2 (b + x)$$

Where a is the concentration of the α form at the beginning, b the concentration of the β form at the beginning and x is the amount of α form which has changed to the β form in the time t, k_1 is the reaction velocity constant for the change of α to β and k_2 is the reaction velocity constant for the change of β to α. The above equation after integration and simplification can be made to take the following form in which the reaction is followed by means of the polariscope.

$$\frac{1}{t} \log \frac{R_o - R}{R_t - R} = k_1 + k_2$$

where R_0 is the rotation at the start R is the rotation after equilibrium has been reached and R_t is the rotation at the time t expressed in hours. For the purpose of comparison K was used in place of $k_1 + k_2$ and common logarithms were used in place of the natural logarithms. This equation is presented in the form used by Hudson (11). Hudson's (10) temperature equation gives 0.475 for the value of K at 25°C. Recalculating some of Trey's (26) data the value for K obtained was 0.478. Verschuur's (29) data gives 0.472 when the rate was determined using α lactose and 0.474 when β lactose was used. We have obtained values in the neighborhood of 0.473 at pH 6.0, at pH 4.5 the value is more nearly 0.460. By dissolving lactose in distilled water some variations in the constant were observed perhaps due to fluctuations in pH of such unbuffered solutions.

In most of our experimental work α lactose was used as the starting material. All rotations were observed at 25°C. using

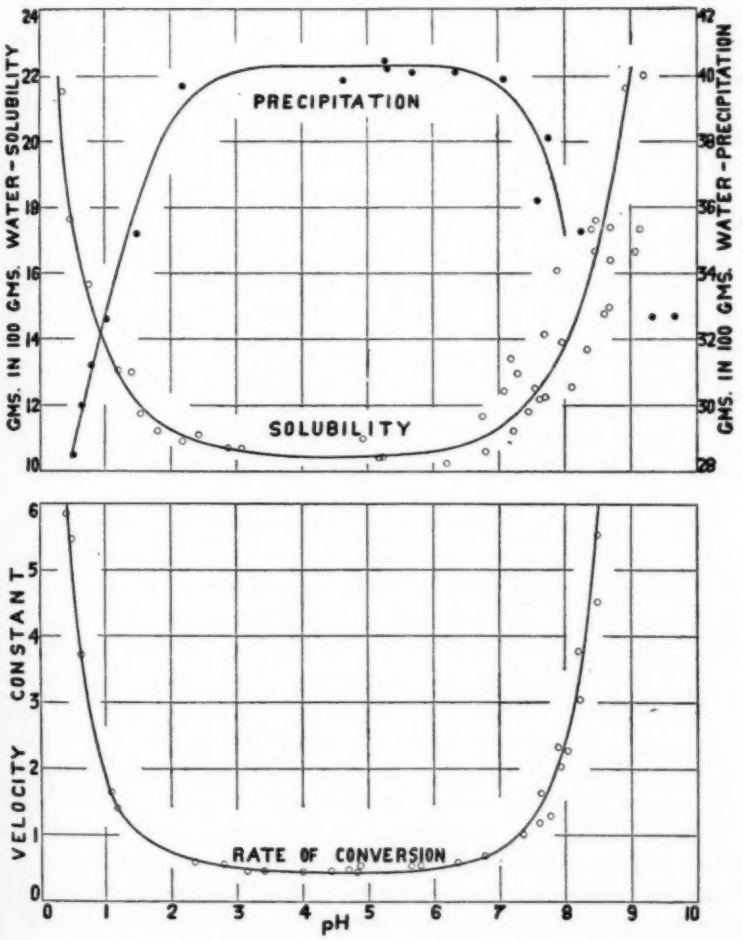

FIG. 1. THE EFFECT OF pH ON THE RATE OF CHANGE OF THE FORMS OF LACTOSE INTO EACH OTHER AS INFLUENCING THE RATE OF SOLUTION AND PRECIPITATION OF LACTOSE

a jacketed 2 decimeter tube. The different pH values were obtained with dilute buffer solutions except in the high acid range where hydrochloric acid alone was used. The results obtained are expressed graphically in figure 1.

This figure shows that within the range which is normally encountered in dairy products, the pH of the solution would exert little effect upon the rate of change of lactose from one form to the other. The curve is fairly symmetrical with a rather wide middle region displaced to the acid side of neutrality, in which a change in pH produces little effect upon the rate of change of the one form of lactose into the other. The rate of change approaches infinity on the acid side at pH 0.0 and on the alkaline side at pH 9.0.

Mills and Hogarth (18) found that milk sugar (α lactose hydrate) has two solubilities which they term "initial" and "permanent" solubility. They show that the initial solubility is obtained at once and is considerably less than the permanent solubility which was obtained only after a long time.

This curve for the effect of the pH of the solution on the rate of change of the two forms of lactose into each other shows that, in the pH region represented by the horizontal portion of the curve, the attainment of the "permanent" solubility of lactose would be slow while at the two ends of the curve where the rate increases sharply the attainment of the permanent solubility of lactose would be rapid. Urech (28) recognized the importance of this change of α lactose hydrate into another form as influencing the rate of solution of lactose. He found that lactose reached its permanent solubility rapidly in acids and alkalies because they accelerated this rate of change. In our experiments this effect was easily demonstrated by shaking solutions of various pH values with an excess of α lactose hydrate at 25°C. for ten minutes, filtering and determining the concentration of lactose in the filtrate. The results obtained in this way are plotted as the "solubility" curve in the upper part of figure 1. It will be observed that this curve is very similar to the rate of change curve in the lower part of figure 1. A more concentrated solution is obtained in a shorter time at the extremes of the curve because solutions of

the corresponding pH values accelerate the rate of change of α into β lactose:

Lactose should crystallize most rapidly from supersaturated solutions in the pH regions where the rate of change of the forms of lactose into each other is most rapid. In two experiments Jenkins (15) showed that the velocity of crystallization of α lactose hydrate was faster in ammonium hydroxide solutions than it was in water. The effect of pH in our experiments was demonstrated by taking 50 cc. of a solution of lactose saturated at 50°C., cooling it down to 25°C., adjusting its pH and adding 5 grams of α lactose hydrate as seeding material. The mixture was then agitated at 25°C. for thirty minutes, at the end of this time the solution was filtered and the concentration of the solution determined. The results obtained are plotted as the "precipitation" curve in the upper part of figure 1. It will be observed that the concentration of the solution decreased most in the regions where the change of the forms of lactose into each other was most rapid. Precipitation in the region of pH 9 is not as rapid as would be expected, indicating the effect of another factor, possibly due to a reaction between the lactose and the alkali. This precipitation curve is the reverse of the solubility curve. A considerable lag in the precipitation of α lactose hydrate was observed even though the solution was thoroughly seeded and agitated continually. There was much less lag in the solubility curve.

The data in figure 1 show that within the range which is normally encountered in dairy products changes in pH will exert little effect on the solution and precipitation of α lactose hydrate in so far as the pH effects the rate of change of the forms of lactose into each other.

The effect of concentrated sucrose solutions on the rate of conversion of the forms of lactose into each other

Ice cream mixtures and condensed milk contain considerable amounts of sucrose. Hunziker and Nissen (14) found that concentrated sucrose solutions decreased the solubility of lactose slightly at 18°C. while Peter (19) found that the solubility of

lactose in a concentrated sucrose solution was appreciably decreased at near the freezing point.

It was conceivable at least that high concentrations of sucrose might affect the rate of change of the forms of lactose into each other. The effect, if any, is however very small. Trey (26) gives data which yield the value for K of 0.478 for the velocity constant in water at 25°C. In his experiment in which 4.5 grams of α lactose and 17.1 grams of sucrose were made up to 100 cc. with water, the value for K was 0.435 and when β lactose was used the value obtained was 0.462. These values are probably all within the limit of error of Trey's experiments.

The average velocity constant of 0.46 was obtained in a number of experiments in which α lactose hydrate was dissolved in water at a pH in the neighborhood of 5.0. When α lactose hydrate was dissolved in about 40 per cent sucrose solutions at the same pH, the values for K obtained in different experiments were 0.454, 0.456, 0.461, 0.458, and 0.461.

It appears that at 25°C. at least, the presence of considerable amounts of sucrose in the solution has little or no effect on the rate of conversion of the two forms of lactose into each other.

Lactose in dried milk

Dried skimmilk is approximately half lactose and consequently one might expect that some of the properties of lactose would be reflected in the properties of the dried milk. Hauser (7) and Hauser and Hering (8) studied the heat of wetting of dried milk of various moisture contents. They found that a break in the curve occured at about 3 per cent moisture content with fresh whole milk powder. They interpreted this break as indicating the place at which the lactose became all hydrated. Different curves were obtained with different types of powders. They recommended the determination of the heat of wetting as a quick method for the estimation of the moisture content of milk powder.

Holm and Greenbank (9) and Supplee (24) have determined the moisture content of dried milk obtained at a series of constant humidities. Holm attributed a difference in moisture content which he found when atmospheric roll and spray dried powders

were held at a constant humidity to a difference in heat treatment of the protein material during the drying process. Supplee discovered a very interesting behavior, namely, that the hydration and dehydration moisture contents of the same powder at the same humidity were quite different. This led him to hold samples of dried milk at humidities in the neighborhood of 50 per cent and he found that samples held at constant humidity at first took up moisture and then gave up a considerable portion of it again.

A study was begun in this laboratory to see if some of these peculiar behaviors of dried milk might not be due to lactose in stead of the proteins as previous investigators were inclined to believe.

Little information is available as to the state of lactose in dried milk. Hauser and Hering (8) say that it is amorphous in the fresh Krause product but indicate that at 3 per cent moisture content it is present as the hydrate. They also mention one powder in which part of the lactose was present as the hydrate and another in which all was present as the hydrate as determined by the polarizing microscope.

We found that by dissolving dried milk in water and then quickly precipitating the proteins with a solution of mercuric chloride and alcohol a clear filtrate containing the lactose in solution was obtained. The filtrate was immediately placed in a polariscope tube and the change in rotation was determined. From these readings the amount of lactose present in the α and β forms was calculated. These readings were all made at 25°C. using a water jacketed polariscope tube.

It was found that the fresh dried skimmilk prepared by the pressure and centrifugal spray and the atmospheric and vacuum drum processes contained approximately the same proportions of α and β lactose as does ordinary fluid skimmilk. The rotation usually fell slightly but this was probably due to the fact that the milk is not dried at 25°C. but at some higher temperature where there is a slightly greater proportion of α lactose as compared with β lactose. This would be expected from the work of Gillis (6) who has shown that the ratio of β/α lactose at equilibrium

varies from 1.65 at 0°C. to about 1.33 at 100°C.; at 25°C. the ratio is about 1.58.

The results obtained by polarizing the solutions prepared from the fresh skimmilk powders listed above indicate that the milk dries before the lactose starts to crystallize or at least dries before any crystillization and change of one form into the other can take place. That the lactose in fresh skimmilk dried by the above mentioned processes is not crystalline was shown by a seeding test which will be described later and by observation with the polarizing microscope.

Skimmilk dried by the flake process when dissolved and subjected to the polariscopic examination described above showed a considerable fall in rotation, indicating that the α hydrate form of lactose had actually crystallized out in this process of drying and that an appreciable amount of β lactose had changed to α lactose before the drying proceeded far enough to stop further change. The presence of α lactose hydrate crystals was confirmed by the seeding test. While possibly not all of the lactose in the α form in the flake process powder is in the crystalline condition yet an amount of α lactose which corresponds to the lactose which has changed from the β to the α form must have necessarily crystallized out. It is probable that most of the α lactose is in the crystalline form in this type of dried milk. The crystallization of lactose in the flake dried skimmilk is started by the continuous seeding of the milk as it enters the drier, due to the mechanics of the way in which the drying is carried out.

Supplee (24) found that the humidity-dehydration curve lies above the humidity-hydration curve when the dried milk was hydrated at 80 per cent humidity.

Our study shows that this result was due in part, at least, to the presence of α lactose hydrate crystals with their 5 per cent of water which would not be removed at any of the humidities used in his dehydration studies.

It was found that concentrations above about 85 per cent sulphuric acid will dehydrate α lactose hydrate crystals at room temperature if given sufficient time. A concentration of about 85 per cent sulfuric acid does not dehydrate α lactose hydrate,

crystals, and yet it removes a considerable amount of moisture from ordinary dried milk. Samples of 4 kinds of dried milk have geen maintained over 85 per cent sulfuric acid for two years and they are still changing in weight. At the present time the moisture contents are: flake 1.97 per cent, pressure spray 1.17 per cent, atmospheric drum 0.96 per cent, and vacuum drum 1.01 per cent. The flake powder has the highest moisture content because of the presence of a considerable amount of α lactose hydrate crystals, the water from which is not removed by 85 per cent sulfuric acid. The range of variation in moisture content of the other 3 kinds of dried milk is 0.21 per cent. This experiment was repeated with an entirely different set of samples with the same result.

Some of the behaviors of dried milk reported by Hauser and Hering (8) can be partially accounted for, at least, on the basis of the crystallization of the lactose. For example, the difference in behavior of dried milk held only a short time and a longer time at the high humidities, and the behavior of dried milk in which the moisture has been increased to a considerable extent and then decreased again, also the differences between the dried milk samples which they cite as containing different amounts of crystalline hydrate.

The lactose in pressure and centrifugally spray dried whey is also present in a non-crystalline equilibrium mixture of α and β lactose.

Theoretically, at least, an indication of the temperature at which the powders are dried can be gained from the ratio of β to α lactose present in the non-crystalline dried material.

Evidence that the lactose in the freshly dried milk by the spray and drum processes is present in the form of a very concentrated syrup seems to be abundant and conclusive. Solution and polarization indicated that the two forms of lactose are in equilibrium. In attempting to dry whey by the roller process a "glass" is obtained on the rolls. Very good evidence that the lactose is not crystalline comes from the humidity equilibrium experiments which will be explained later. The absence of α lactose hydrate crystals in spray and roller dried milk and in spray dried whey is shown by a seeding test, and by the polarizing microscope.

Lactose forms fairly stable supersaturated solutions in the absence of seeding nuclei. A solution of lactose saturated at 50°C. was filtered and cooled to 20°C. Ten cubic-centimeter portions of this solution were placed in test tubes and small amounts of the dried milks (about 25 mgm.) being tested were added to each tube. The tubes were stoppered and shaken at intervals for about one hour. During this time the solution of lactose will begin to crystallize if seeding crystals of α lactose hydrate are present in the material added. The fresh spray and drum dried skimmilk and the spray dried whey show no crystallization of the solution when this test is applied but flake dried skimmilk induces copious crystallization. Spray and drum dried milk powders of the various kinds as well as spray dried whey show copious crystallization when this test is applied provided they have once definitely caked. The seeding test was previously described by Hudson and Brown (12) for detecting the presence of α lactose hydrate in β lactose preparations.

Prof. C. W. Mason was kind enough to examine a series of dried milk and dried whey samples for crystallization, with the polarizing microscope. His observations as to the presence or absence of crystals were a complete confirmation of the results obtained by the seeding test with the exception that occasionally a crystal of some material was observed in samples which gave a negative seeding test at the end of one hour.

The caking of dried milk

Schmoeger (22) reported that if a pure lactose solution was dried very rapidly a non-crystalline "glass" was obtained which took up moisture very readily, became sticky and then crystallized to a hard mass of α hydrate lactose.

Supplee (24) has shown that in the neighborhood of 50 per cent relative humidity dried milk will first take up moisture and then give up some of it again. We have confirmed his observation and shown that dried whey also does the same thing. After these products have taken up moisture and then given it up again it is found that they have caked. Since the lactose in dried milk and whey is present in the form of a very concentrated syrup it

consequently produces a solution with a very low vapor pressure or very high osmotic pressure. This solution of lactose is so concentrated that the lactose cannot crystallize and if the moisture content of the milk powder is maintained at a low enough level crystallization will not occur and the milk powder does not cake. Since such a concentrated syrup is very hygroscopic it tends to take up water when exposed to atmospheres which con-

TABLE 1

Distribution of the two forms of lactose in dried milk after storage for five months at 70 per cent humidity

KIND OF DRIED MILK	PREVIOUS TREATMENT	LACTOSE		β WHICH HAS BEEN CHANGED TO α	SEEDING TEST	POLARIZING MICROSCOPE, CRYSTALS
		α	β			
		per cent	*per cent*	*per cent*		
Open roll	Fresh	(38.7)	(61.3)	0	−	−
	5 months at 70 per cent humidity	53.1	46.9	23.5	+	+
Pressure spray	Fresh	(38.7)	(61.3)	0	−	−
	5 months at 70 per cent humidity	51.1	48.9	20.2	+	+
Vacuum roll	Fresh	(38.7)	(61.3)	0	−	−
	5 months at 70 per cent humidity	50.0	50.0	18.4	+	+
Flake	Fresh	63.0	37.0	39.6	+	+
	5 months at 70 per cent humidity	77.8	22.2	63.8	+	+

tain appreciable moisture, this water which the dried milk takes up dilutes the lactose solution tending to cause the milk particles to stick together and if enough water is taken up in this way the lactose solution is diluted to the concentration where it can crystallize. After crystallization begins the concentration of the solution is decreased due to the solid phase separating out; this decreases the osmotic pressure and increases the vapor tension causing some of the moisture to be given up again. This lactose which crystallizes out is, of course, α hydrate and crystallizes with a molecule of water. The crystallization causes a caking of

the product. In the case of dried whey in which this process can be followed with ease, the material may become plastic and when crystallization occurs the product becomes quite hard.

The change of β to α lactose does not reach completion except possibly after a very long time. Samples have been under observation for two years and they are still changing. This is perhaps due to slow diffusion through the nearly solid syrup. Some idea of the extent of this change is given in table 1. This table shows the change of β to α lactose in milk powders held for about five months at a humidity of approximately 70 per cent. These powders first took up moisture and then gave up some of it again.

The fresh samples of open and vacuum roll and spray powders are assumed to be in equilibrium with a ratio of 1.58 parts of β to 1.0 part of α. Actually the rotation of such fresh powders may indicate a slightly higher proportion of α lactose but the fall in rotation is only a few hundredth of a degree. The seeding tests have been negative.

Crystallization of lactose in ice cream

Bothell (2) and Zoller and Williams (31) showed that sandy ice cream was caused by the crystallization of lactose hydrate. Sharp (23) presented an explanation for the crystallization and development of α lactose hydrate crystals in ice cream based on the assumption that both α lactose hydrate and β lactose anhydride crystallized out and that the growth of the α lactose hydrate crystals was controlled by the effect of temperature on the rate of change of β to α lactose. Although this explanation seemed to agree with the general development of "sandy" ice cream, it was shown to be incorrect by the very simple seeding test. Ice cream may be held for a very appreciable period and yet be free from crystals of α lactose hydrate which induce copious crystallization of a supersaturated lactose solution. Sandy ice cream does not produce such clear cut seeding action on a supersaturated solution of lactose as do some other milk products.

The amounts of α and β lactose in ice cream can be determined by means of the polariscope using the same procedure as was described for dried milk. It was found that in ice cream which

was not sandy, the ratio of β to α lactose was undisturbed and was approximately the same as in a solution of lactose at equilibrium at room temperature. The change obtained in the optical rotation was hardly detectable. In the case of sandy ice cream, however, there was always an excess of α, the excess increasing with the degree of sandiness. In one instance where a case of incipient sandiness was examined the excess of α was about six times the maximum allowable error in reading the polariscope, showing that sandiness can probably be detected and followed by means of the polariscope long before it can be detected organolyptically. Only two out of several experienced judges recognized the sandiness of this sample and then only after repeated trials.

The polariscopic method of following the development of sandiness in ice cream has distinct advantages. Any method of attempting to filter off the crystallized lactose from the freshly melted ice cream has the error that some of the lactose will go into solution before the separation can be accomplished. In using the polariscopic method, however, the lactose is all put back into solution and the excess of α determined from the polariscope readings.

The crystallization of α lactose hydrate in ice cream probably follows a course similar to the crystallization of α lactose hydrate in dried milk. The solubility limit of α lactose hydrate must be exceeded in order for it to crystallize in ice cream. The concentration of the lactose solution is accomplished by the freezing of water. A concentration of lactose in the unfrozen water of the ice cream exists where the α lactose hydrate is most susceptible to crystallization, if the solution in the ice cream becomes too concentrated crystallization cannot occur because not enough water is present to permit the lactose molecules to arrange themselves in a definite grouping which is necessary for crystal formation. Ordinarily the ice cream is drawn from the freezer and placed in a cold hardening room. The property of lactose by which it forms supersaturated solutions is an important factor. The freezing of water during the hardening process may concentrate the lactose solution until it cannot crystallize. Crystallization is made more difficult because of the mixture of three kinds

of sugar molecules in the syrup, namely sucrose, β lactose, and α lactose. If the ice cream is warmed to some extent ice melts and dilutes the syrup, this dilution may be to such an extent that the α lactose hydrate can crystallize. The conditions under which the crystallization first starts are probably influenced by seeding nuclei.

The forms of lactose in the dried residue in the total solids determination

Schmoeger (22) Hudson (12) Rice and Miscall (20) Hunziker and Nissen (14) and Koestler and Lörtscher (16) seem to be fairly well in agreement that the lactose in the dried residue in the total solids determination in milk is present as the anhydride. With the exception of Hudson (12) and Schmoeger, no clear indication is given as to which anhydride was meant. Schmoeger dried milk on sand, extracted the sand with a small amount of water, precipitated the proteins with lead acetate and polarized the filtrate. He found that the rotation decreased only slightly on standing. He concluded from this result that the lactose was present in the dried residue as a mixture of the two forms which we know as α and β lactose.

We have also found that the lactose is present in the dried residue from the total solids determination, as oridnarily made, as the equilibrium mixture of β and α lactose. Skimmilk was dried according to the method for determining total solids, (*a*) using the Mojonnier method, (*b*) by drying in a thin layer in a boiling water oven, and (*c*) by drying in a thin layer at 50°C. In all three cases the lactose in the residue was found to be the equilibrium mixture of α and β. When relatively large bulks of milk were preserved with mercuric chloride and dried slowly at 50°C. α lactose hydrate crystallized out, disturbing the equilibrium until eventually practically all of the lactose crystallized out as the α hydrate form.

These experiments indicate that the lactose in the residue in the total solids determination is present in a non-crystalline condition as a concentrated syrup which has become so concentrated that in ordinary analytical work it cannot be distinguished from the

anhydrides. If it were true that the α lactose hydrate crystallized out in the total solids determination of milk it would probably give a different value for the total solids depending upon the extent to which the water was removed from the crystalline α lactose hydrate.

SUMMARY AND CONCLUSION

1. Between the pH values of 2.0 and 7.0 the rate of change of α and β lactose into each other is at a minimum. The rate approaches infinity at pH 0.0 and 9.0.

2. The effect of pH on the rate of change of α to β lactose was shown to influence the rate of solution of α lactose hydrate and the effect of pH on the rate of change of β to α lactose was shown to influence the rate of precipitation of α lactose hydrate at 25°C.

3. Relatively concentrated sucrose solutions have little effect on the rate of change of the two forms of lactose into each other at 25°C.

4. Lactose was found to be in a non-crystalline equilibrium mixture of the α and β forms in skimmilk dried by the pressure and centrifugal spray and by the atmospheric and vacuum drum methods, when freshly prepared and maintained at a low moisture content. A considerable amount of α lactose hydrate was found to have crystallized in skimmilk during drying by the flake process.

5. The caking of milk powder is believed to proceed as follows: (1) the absorption of moisture by the concentrated lactose syrup, (2) the adherence of the milk particles to one another, and (3) the solidification of the mass due to the crystallization of some of the lactose as α hydrate.

6. The formation of "sandy" ice cream was explained as being due to the presence of sufficient non-frozen water to permit the α lactose hydrate to crystallize. By rapidly freezing out the water from ice cream, the lactose solution may be concentrated to a region where the crystallization of the α lactose hydrate does not occur. α lactose hydrate crystals form in such ice cream if sufficient ice is melted to dilute the syrup to a range of concentration where crystallization can take place.

7. The Hudson and Brown seeding test was shown to be useful

for recognizing the presence of crystalline α lactose hydrate in dairy products.

8. Lactose was found to be present in the dry residue of the total solids determination in milk as an equilibrium mixture of α and β lactose in the non-crystalline condition.

9. It was found that nearly all of the lactose crystallized out as the α hydrate form when milk was dried very slowly at 50°C.

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